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Your last SELECT statement was:
   S MGN417 OR (MGN (2N) 417)

Ref        Items    File
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N1            14    545: Investext(R)_1982-2001/May 18
N2             1    349: PCT Fulltext_1983-2001/UB=20010510, UT=20010426
N3             0      2: INSPEC_1969-2001/May W2
N4             0      5: Biosis Previews(R)_1969-2001/May W2
N5             0      6: NTIS_1964-2001/Jun W1
N6             0      8: Ei Compendex(R)_1970-2001/May W2
N7             0      9: Business & Industry(R)_Jul/1994-2001/May 17
N8             0     10: AGRICOLA_70-2001/May
N9             0     14: Mechanical Engineering Abs_1973-2001/Mar
N10            0     15: ABI/Inform(R)_1971-2001/May 18
   2 files have one or more items; file list includes 287 files.

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?b n2 n1;exs
       18may01 14:13:47 User228206 Session D1488.2
            $1.86    1.489 DialUnits File411
     $1.86  Estimated cost File411
     $0.10  TYMNET
     $1.96  Estimated cost this search
     $1.97  Estimated total session cost   1.663 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File 349:PCT Fulltext  1983-2001/UB=20010510, UT=20010426
         (c) 2001 WIPO/MicroPat
  File 545:Investext(R)  1982-2001/May 18
         (c) 2001 Thomson Financial Networks

      Set  Items  Description
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Executing TD315
>>>SET HILIGHT: use ON, OFF, or 1-5 characters
               0  MGN417
           21078  MGN
          313449  417
              15  MGN(2N)417
      S1      15  MGN417 OR (MGN (2N) 417)
?t s1/kwic/1
```

1/KWIC/1   (Item 1 from file: 349)

DIALOG(R)File 349:(c) 2001 WIPO/MicroPat. All rts. reserv.

Fulltext Availability:
  Detailed Description

Detailed Description

... S. typhimutium host MGN-392 containing expression vector pMEG-104, the combination designated as strain **MGN -417** . pMEG-104 contains the asd gene of S. typhimurium operatively linked to the promoter left...of pMEG-096 in MGN-377 leaving the cI857PRc2 cartridge in the defined asd deletion.

MGN -417 69836 6/6/95 S. typhimurium UK-1 ELVS host MGN-392 (pMEG-104 P22P...to produce MGN-401 (Table 1), or the ELVS expression vector pMEG-104, to produce **MGN -417** (Table 3). **MGN** -336 was electroporated with the ELVS expression vector pMEG-104, to produce MGN-409 (Table...

...hours. The plating efficiency, measured as colony forming units, observed at room temperature (25C) for **MGN -417** is approximately 10,000 less than that observed for MGN-401, containing the Asd- vector...

...extremely small, indicating poor growth survival (Table 4). The

updated search 2001 18

reduction obtained in plating efficiency of **MGN -417** at 25'C in the presence ...further demonstrated by the data in Figure 6 for the Salmonella based ELVS in which **MGN -417** (Table 3) fails to grow m complex liquid media at room temperature. Under these conditions, **MGN - 417** is unable to proliferate and exhibits a reduction in recoverable bacteria over a 24 hour...

?t s1/3/1

1/3/1 (Item 1 from file: 349)

DIALOG(R)File 349:PCT Fulltext

00445018

RECOMBINANT BACTERIAL SYSTEM WITH ENVIRONMENTALLY LIMITED VIABILITY

SYSTEME BACTERIEN RECOMBINE A VIABILITE LIMITEE PAR L'ENVIRONNEMENT

Patent Applicant/Assignee:
  WASHINGTON UNIVERSITY
Inventor(s):
  CURTISS Roy III
  TINGE Steven A
Patent and Priority Information (Country, Number, Date):
  Patent: WO 9640947 A1 19961219
  Application: WO 96US9774 19960607 (PCT/WO US9609774)
  Priority Application: US 95473789 19950607
Designated States: AU CA CN HU IL JP MX NO NZ PL RU UA AT BE CH DE DK ES FI FR GB GR IE IT NL PT SE
Publication Language: English
Fulltext Word Count: 25180

?t s1/kwic/2-15

Fusions of bacteriophage P22 late genes to the Escherichia coli lacZ gene.

Do Not Copy

Riggs PD; Botstein D
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.

Journal of virology (UNITED STATES) Nov 1987, 61 (11) p3621-4, ISSN 0022-538X Journal Code: KCV
Contract/Grant No.: GM18973, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 8802
Subfile: INDEX MEDICUS

The late genes of bacteriophage P22 were fused to lacZ to study their differential expression from the late operon transcript. No instances of posttranscriptional regulation were uncovered, thus supporting the model that the late genes are expressed, by and large, in fixed ratios based on their translational efficiency and message stability.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Cloning, Molecular; *Escherichia coli--Genetics--GE; *Genes, Bacterial; *Genes, Structural; *Genes, Viral; ***Salmonella** typhimurium --Genetics--GE; * **Salmonella** Phages--Genetics--GE; *Viral Proteins --Genetics--GE; Alleles; DNA Restriction Enzymes; Genotype

CAS Registry No.: 0 (Viral Proteins); 0 (Viral Tail Proteins)

Enzyme No.: EC 3.1.21 (DNA Restriction Enzymes)

DIALOG West Searches 8/00

A quantitative model for nonrandom generalized transduction, applied to the phage P22- Salmonella **typhimurium system.**

Mandecki W; Krajewska-Grynkiewicz K; Klopotowski T

Genetics (UNITED STATES) Oct 1986, 114 (2) p633-57, ISSN 0016-6731
Journal Code: FNH
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 8702
Subfile: INDEX MEDICUS

A mathematical model for nonrandom generalized transduction is proposed and analyzed. The model takes into account the finite number of transducing particle classes for any given marker. The equations for estimation of the distance between markers from contransduction frequency data are derived and standard errors of the estimates are given. The obtained relationships depend significantly on the number of classes of transducing fragments. The model was applied to estimate the number of transducing fragment classes for a given marker in transduction with phage P22 of **Salmonella** typhimurium. It was found that the literature data on frequencies of contransduction in crosses with mutual substitution of selective and nonselective markers can be rationalized most accurately by assuming that the mean number of classes is equal to 2. An improved method for analysis of cotransduction data is proposed on the basis of our model and the results of calculation. The method relies on solving a set of algebraic equations for cotransduction frequencies of markers located within one phage length. The method allows a relatively precise determination of distances between markers, positions of transducing particle ends and deletion or insertion lengths. The approach is applied to the trp-cysB-pyrF and aroC-hisT-purF-dhuA regions of the **Salmonella** typhimurium chromosome.

bacteriophage P22 heads it was possible to isolate cosmid recombinants that could complement the aroD mutation of CU038 either by in vitro selection using minimal medium or in vivo selection for restoration of virulence in BALB/c mice. Repackaged P22 cosmid banks could provide a simple system for selecting in vivo for **Salmonella** virulence determinants. A **Salmonella** typhi strain harbouring mutations in aroA and aroD was constructed for potential use as a live oral typhoid vaccine in humans.
Tags: Animal; Male
Descriptors: Cosmids; *Genes, Bacterial; * **Salmonella** typhimurium --Genetics--GE; **Salmonella** Phages--Genetics--GE; *Transduction, Genetic ; Bacteriophage lambda--Genetics--GE; Mice; Mice, Inbred BALB C; Mice, Inbred Strains; **Salmonella** typhimurium--Pathogenicity--PY; **Salmonella** Infections--Microbiology--MI; Virulence
CAS Registry No.: 0 (Cosmids)

2/9/117 (Item 117 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05563751 89348017
Nucleotide sequence of the bacteriophage P22 **gene 19 to 3 region: identification of a new gene required for lysis.**
Casjens S; Eppler K; Parr R; Poteete AR
Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City 84132.
Virology (UNITED STATES) Aug 1989, 171 (2) p588-98, ISSN 0042-6822
Journal Code: XEA
Contract/Grant No.: 1 U41RR-01685, RR, NCRR; GM21975, GM, NIGMS; AI24083, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 8911
Subfile: INDEX MEDICUS
The nucleotide sequence of a 2558-bp region of bacteriophage P22 at the right end of the genetic map between genes 19 and 3 was determined. A new gene that is partially required for lytic growth, named gene 15, was noted. P22 mutants were constructed which lack gene 15 function, and the gene 15 product was found to be required for lysis in the presence of some divalent cations. It has extensive amino acid sequence similarity with the phage lambda Rz gene, which has a similar function, and weak similarity to the phage T7 18.5 gene which previously had no known function. A hybrid P22 phage, in which the T7 18.5 gene replaces the P22 gene 15, exhibits the plating properties of wild-type P22, strongly suggesting that the two genes have similar functions. In addition, deletions were constructed which show that phage P22 has no additional genes required for lytic growth of lysogeny between genes 19 and 3.
Tags: Comparative Study; Support, U.S. Gov't, P.H.S.
Descriptors: DNA, Viral--Genetics--GE; *Genes, Viral; * **Salmonella** Phages--Genetics--GE; *Viral Proteins--Genetics--GE; Amino Acid Sequence; Base Sequence; Cations, Divalent; Cloning, Molecular; Molecular Sequence Data; Protein Conformation; Restriction Mapping; Viral Proteins--Physiology --PH; Viral Proteins--Ultrastructure--UL
Molecular Sequence Databank No.: GENBANK/J04356
CAS Registry No.: 0 (Cations, Divalent); 0 (DNA, Viral); 0 (Viral Proteins); 126469-21-4 (gene 15 protein, bacteriophage p22)

2/9/123 (Item 123 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05275196 88084352
A novel P22 **prophage in** Salmonella **typhimurium.**
Downs DM; Roth JR
Department of Biology, University of Utah, Salt Lake City 84112.
Genetics (UNITED STATES) Nov 1987, 117 (3) p367-80, ISSN 0016-6731

1733600 71114652
Immunogenetic studies on microorganisms. 32. Integration site of prophage P22 and genetic locus of O-20 in Salmonella **chromosome]**
Kishi K
Igaku to seibutsugaku (JAPAN) Dec 10 1970, 81 (6) p311-4, ISSN 0019-1604 Journal Code: LPA
Languages: JAPANESE
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7105
Subfile: INDEX MEDICUS
Descriptors: Antigens; *Chromosomes, Bacterial; ***Salmonella** Phages; Epitopes

2/9/315 (Item 315 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01652756 74304795
Mutant of Salmonella **typhimurium that channels infecting bacteriophage P22 toward lysogenization.**
Tokuno SI; Goldschmidt EP; Gough M
Journal of bacteriology (UNITED STATES) Aug 1974, 119 (2) p508-13, ISSN 0021-9193 Journal Code: HH3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7412
Subfile: INDEX MEDICUS
Descriptors: Lysogeny; *Mutation; ***Salmonella** typhimurium--Growth and Development--GD; * **Salmonella** Phages--Growth and Development--GD; Cyclic AMP--Biosynthesis--BI; Drug Resistance, Microbial; Mutagens; Nitrosoguanidines; Polymyxin--Pharmacology--PD; **Salmonella** typhimurium --Drug Effects--DE; **Salmonella** typhimurium--Metabolism--ME

2/9/316 (Item 316 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01494461 75082043
Suppressor-dependent frameshift mutants of bacteriophage P22.
Uomini JR; Roth JR
Molecular & general genetics (GERMANY, WEST) 1974, 134 (3) p237-47, ISSN 0026-8925 Journal Code: NGP
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7505
Subfile: INDEX MEDICUS
Descriptors: Mutation; * **Salmonella** Phages; Chromosome Aberrations; Chromosome Mapping; Chromosomes, Bacterial; **Salmonella** typhimurium; Suppression, Genetic
?t s2/9/327 334 352 363 369 376

2/9/327 (Item 327 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01480210 74145283
Control of transcription in phage P22 **infected host.**
Chakravorty M; Khandekar PS; Rao GR; Taneja S
Basic life sciences (UNITED STATES) 1974, 3 p35-52, ISSN 0090-5542 Journal Code: 9KO
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7408
Subfile: INDEX MEDICUS
Descriptors: **Salmonella** typhimurium--Metabolism--ME; * **Salmonella**

Phages--Metabolism--ME; *Transcription, Genetic; Bacterial Proteins --Biosynthesis--BI; Bacteriolysis; Centrifugation, Density Gradient; Chromosome Mapping; DNA, Bacterial--Metabolism--ME; DNA, Viral--Metabolism --ME; Genes; Genes, Regulator; Lysogeny; Muramidase--Biosynthesis--BI; Mutation; Nucleic Acid Hybridization; Phosphorus Radioisotopes; RNA, Bacterial--Biosynthesis--BI; RNA, Viral--Biosynthesis--BI; **Salmonella** Phages--Enzymology--EN; Tritium; Uridine--Metabolism--ME; Viral Proteins --Biosynthesis--BI; Virus Replication

2/9/334 (Item 334 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01461332 73145194
A component mutation of virB virulence affects lysogenization by phage P22.
Bronson MJ; Levine M
Virology (UNITED STATES) Feb 1973, 51 (2) p504-5, ISSN 0042-6822
Journal Code: XEA
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7307
Subfile: INDEX MEDICUS
Descriptors: DNA Viruses--Growth and Development--GD; *Lysogeny; *Mutation; * **Salmonella** typhimurium; * **Salmonella** Phages--Growth and Development--GD; *Virulence; Cell Survival; Genes, Regulator; Genetic Complementation Test; **Salmonella** typhimurium--Growth and Development--GD

2/9/352 (Item 352 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01446913 72160058
Cis-trans position effect in Salmonella **phage** P22.
Kolstad R; Enquist L
Molecular & general genetics (GERMANY, WEST) 1972, 115 (1) p19-25, ISSN 0026-8925 Journal Code: NGP
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7208
Subfile: INDEX MEDICUS
Descriptors: Genes; * **Salmonella** typhimurium; ***Salmonella** Phages; Chromosome Mapping; Crosses, Genetic; Genes, Structural; Genetic Complementation Test; Genetics, Microbial; Mutation; Operon

2/9/363 (Item 363 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01035650 72031168
Transfection of Escherichia coli and Salmonella **typhimurium spheroplasts: host-controlled restriction of infective bacteriophage** P22 **deoxyribonucleic acid.**
Benzinger R; Kleber I
Journal of virology (UNITED STATES) Aug 1971, 8 (2) p197-202, ISSN 0022-538X Journal Code: KCV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7202
Subfile: INDEX MEDICUS
Descriptors: DNA, Viral; *Escherichia coli; *Protoplasts; ***Salmonella** typhimurium; * **Salmonella** Phages; *Transformation, Genetic; Centrifugation, Density Gradient; Cesium; Chlorides; DNA, Viral--Isolation and Purification--IP; Edetic Acid; Muramidase; Penicillinase; Penicillins;

Precipitation; Protamines--Pharmacology--PD; **Salmonella** Phages--Isolation and Purification--IP; Sulfates--Pharmacology--PD; Transformation, Genetic --Drug Effects--DE

2/9/369 (Item 369 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

00984583 72055876
Delayed lysis in Salmonella **phage** P22: **the continued division of mutant-infected cells actively producing phaage.**
Cohen LW; Showers MR; Andrus WD
Virology (UNITED STATES) Sep 1971, 45 (3) p848-52, ISSN 0042-6822
Journal Code: XEA
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7203
Subfile: INDEX MEDICUS
Descriptors: Cell Division; *Lysogeny; *Mutation; * **Salmonella** typhimurium--Growth and Development--GD; ***Salmonella** Phages--Growth and Development--GD; Culture Media; DNA, Bacterial--Biosynthesis--BI; Manometry ; Microscopy, Phase-Contrast; Mitomycins--Pharmacology--PD; Oxygen Consumption; **Salmonella** typhimurium--Drug Effects--DE; **Salmonella** typhimurium--Metabolism--ME; Time Factors; Virus Replication

2/9/376 (Item 376 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

00981737 72012276
Synthesis and maturation of phage P22 **DNA. II. Properties of temperature-sensitive phage mutants defective in DNA metabolism.**
Botstein D; Levine M
Journal of molecular biology (ENGLAND) Jun 28 1968, 34 (3) p643-54, ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 7201
Subfile: INDEX MEDICUS
Descriptors: DNA, Viral--Analysis--AN; **Salmonella** Phages--Analysis--AN ; Centrifugation, Density Gradient; Deoxyribonucleases; DNA, Bacterial --Analysis--AN; DNA, Bacterial--Metabolism--ME; DNA, Viral--Biosynthesis --BI; DNA, Viral--Metabolism--ME; Genetics, Microbial; Mutation; Nucleic Acid Hybridization; **Salmonella** typhimurium--Analysis--AN; **Salmonella** Phages--Metabolism--ME; Temperature; Thymidine--Metabolism--ME; Tritium
?t s2/9/418 446 461 537 584 583 585 586

2/9/418 (Item 418 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

00905460 66124255
Transduction of Escherichia coli genetic material by Phage P22 **in** Salmonella **typhimurium x E. coli hybrids.**
Eisenstark A
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 1965, 54 (6) p1557-60, ISSN 0027-8424
Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 6609
Subfile: INDEX MEDICUS
Tags: In Vitro
Descriptors: Coliphages; *Genetics; * **Salmonella** typhimurium; * **Salmonella** Phages; Mutation

0062807 DBA Accession No.: 87-07155

Cloning vectors derived from phage P22 **- cloning in Escherichia coli**

AUTHOR: Moyle H; Youderian P

CORPORATE SOURCE: Department of Biological Sciences, USC, Los Angeles, CA, USA. (Meet., 191) 1986

CODEN: 9999Z

LANGUAGE: English

ABSTRACT: 3 Phage and cosmid cloning vectors derived from phage P22 were constructed. The first was a P22 phage carrying cos, StrR SpcR determinants, and the pSC101 origin, suitable for cloning 15 to 20 kb fragments of **Salmonella** DNA. The second was a P22 phage carrying cos, StrR SpcR determinants, the pSC101 origin, and the lac operon mutated by substitution of a frameshifted polylinker at the beginning of lacZ (designed to replace lambda-gt11). cDNA clones may be selected as Lac+ transfectants following packaging and adsorption to a heteroimmune lac-delta Escherichia coli host, requiring that insertions be translated in frame with the C-terminus of lacZ. The third vector is part of a cosmid system that allows cloning of 50 kb inserts into oversized P22 prophages, and may permit chromosomal walking by a simple recombination test. The vectors may be propagated as P22 phages to obtain high yields of stable particles. Packaged clones may be directly selected in Escherichia coli. (0 ref)

DESCRIPTORS: phage P22 der. vector construction, cloning in Escherichia coli, appl. bacterium

SECTION: Microbiology-Genetics (A1)

2/9/586 (Item 6 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs

0012763 DBA Accession No.: 83-05475

Transformation with plasmid DNA in Salmonella **typhimurium- transformation with plasmid pBR322 and phage** P22 **(conference abstract)**

AUTHOR: MacLachlan P R; Sanderson K E

CORPORATE SOURCE: Department of Biology, University of Calgary, Canada.

JOURNAL: Abstr.Can.Soc.Microbiol. (32 Meet.. 81)

CODEN: 0006

2/9/537 (Item 4 from file: 76)

DIALOG(R)File 76:Life Sciences Collection

01277068 2056488

Genetic structure of the bacteriophage P22 **P sub(L) operon.**

Semerjian, A.V.; Malloy, D.C.; Poteete, A.R.

Dep. Mol. Genet. and Microbiol., Univ. Massachusetts, Worcester, MA 01655, USA

J. MOL. BIOL. vol. 207, no. 1, pp. 1-13 (1989.)

DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH

SUBFILE: Microbiology Abstracts Section B: Bacteriology; Biochemistry Abstracts Part 2: Nucleic Acids; Genetics Abstracts; Virology Abstracts

The sequence of 1416 base-pairs of the P22 P sub(L) operon was determined, linking a continuous sequence from P sub(L) through abc2). P22 mutants bearing deletions in the sequenced region were constructed and tested for their phenotypes. Plasmids were constructed to express P sub(L) operon genes singly and in combination from P sub(lac) UV5. Two previously known genes, 17 and c3, are located within this sequence. In addition, three new genes have been identified: ral, kil and arf .

DESCRIPTORS: phage P22; **Salmonella** typhimurium

IDENTIFIERS: PL gene; genes; nucleotide sequence; ral gene; kil gene; arf gene

SECTION HEADING: 02750 --Phage-host interactions; 14640 --Structure & sequence; 07311 --Phages-initial names; 22050 --Viral genetics including virus reactivation

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      $2.94  Estimated cost this search
      $5.19  Estimated total session cost   2.398 DialUnits


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### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 3106900061...Open

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PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
### Status: Signing onto Dialog
 ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
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### Status: Connected


Dialog level 00.07.20D

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         (c) format only 2000 Dialog Corporation
  File 654:US Pat.Full.  1990-2000/Jul 25
         (c) format only 2000 The Dialog Corp.
*File 654: Reassignment data current through 12/06/1999 recordings.
Due to recent processing problems, the SORT command is not working.
  File 349:PCT Fulltext  1983-2000/UB=, UT=20000713
         (c) 2000 WIPO/MicroPatent
  File 148:Gale Group Trade & Industry DB  1976-2000/Jul 28
         (c)2000 The Gale Group
  File 649:Gale Group Newswire ASAP(TM)  2000/Jul 28
         (c) 2000 The Gale Group
  File 813:PR Newswire  1987-1999/Apr 30
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  File  16:Gale Group PROMT(R)  1990-2000/Jul 28
         (c) 2000 The Gale Group
  File  34:SciSearch(R) Cited Ref Sci  1990-2000/Jul W4
         (c) 2000 Inst for Sci Info
  File  76:Life Sciences Collection  1982-2000/May
         (c) 2000 Cambridge Sci Abs
  File 154:MEDLINE(R)  1993-2000/Sep W4
         (c) format only 2000 Dialog Corporation
  File 348:European Patents  1978-2000/Jul W01
         (c) 2000 European Patent Office
*File 348: Update 200026 was withdrawn July 26 due to data problems.
Corrected data will be online sometime in the next few days.
  File 440:Current Contents Search(R)  1990-2000/Aug W1
         (c) 2000 Inst for Sci Info
  File 621:Gale Group New Prod.Annou.(R)  1985-2000/Jul 28
         (c) 2000 The Gale Group
  File 636:Gale Group Newsletter DB(TM)  1987-2000/Jul 28
         (c) 2000 The Gale Group
  File 653:US Patents Fulltext  1980-1989
         (c) format only 2000 The Dialog Corp.
*File 653: Reassignment data current through 12/06/1999 recordings.
Due to recent processing problems, the SORT command is not working.
  File   5:Biosis Previews(R)  1969-2000/Jul W4
         (c) 2000 BIOSIS
```

Hybrid hepatitis B virus core-pre-S proteins synthesized in avirulent Salmonella **typhimurium and** Salmonella **typhi for oral vaccination.**

Schodel F; Kelly SM; Peterson DL; Milich DR; Curtiss R 3rd
Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Infection and immunity (UNITED STATES) May 1994, 62 (5) p1669-76, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: AI20702, AI, NIAID; AI33562, AI, NIAID; DE06669, DE, NIDCR
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9408
Subfile: INDEX MEDICUS

Avirulent salmonellae expressing foreign genes are attractive for use as oral vaccine carriers. To facilitate the stable expression of heterologous genes without conferring antibiotic resistance, a deletion of the asdA1 gene was introduced into Salmonella typhimurium and S. typhi delta cya delta crp mutant vaccine strains. An **asd** -complementing plasmid expressing hybrid hepatitis B virus nucleocapsid-pre-S (HBcAg-pre-S) particles was constructed. These hybrid HBcAg-pre-S particle genes were stably expressed in S. typhimurium and S. typhi delta cya delta crp mutant vaccine strains in this balanced, lethal host-vector combination. A single oral immunization of BALB/c mice with a recombinant S. typhimurium delta cya delta crp mutant synthesizing hybrid HBcAg-pre-S elicited potentially virus-neutralizing anti-pre-S serum immunoglobulin G antibodies. In addition, serum immunoglobulin G recognizing S. typhimurium lipopolysaccharide was induced. Distribution in tissue after oral immunization was analyzed in one plasmid-strain combination. The recombinant S. typhimurium colonized the gut-associated lymphoid tissue and the spleen and persisted for over 4 weeks, retaining the HBcAg-pre-S expression plasmid. An isogenic virulence plasmid-cured S. typhimurium delta cya delta crp strain expressing the same HBcAg-pre-S gene had reduced immunogenicity for the carried antigen after oral immunization.

Tags: Animal; Female; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Expression of a recombinant Entamoeba histolytica antigen in a Salmonella typhimurium vaccine strain.

Cieslak PR; Zhang T; Stanley SL Jr
Department of Medicine, Washington University School of Medicine, St Louis, MO 63110.

Vaccine (ENGLAND) 1993, 11 (7) p773-6, ISSN 0264-410X
Journal Code: X6O
Contract/Grant No.: 5T32AI07172, AI, NIAID; AI30084, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9311
Subfile: INDEX MEDICUS

The expression of a major surface antigen of the intestinal protozoal parasite Entamoeba histolytica in an attenuated Salmonella typhimurium vaccine strain is described. A polymerase chain reaction fragment derived from cDNA encoding the serine-rich Entamoeba histolytica protein, SREHP, was introduced into S. typhimurium chi 3987 (delta cya delta crp delta **asd**) using a plasmid expression vector (pYA292) containing the aspartate semialdehyde (**asd**) gene. S. typhimurium expressing recombinant SREHP as a SREHP/maltose binding protein fusion protein was administered orally to mice and gerbils (an important animal model for E. histolytica infection) and was recovered from splenic tissue in both species. Our study demonstrates the feasibility of expressing recombinant amoebic proteins in attenuated S. typhimurium strains, and shows that vaccine strains of S. typhimurium can successfully infect the gerbil, a widely used model for amoebic liver abscess and intestinal amoebiasis.

Tags: Animal; Female; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Expression of a recombinant Entamoeba histolytica antigen in a Salmonella **typhimurium vaccine strain.**

Cieslak PR; Zhang T; Stanley SL Jr
Department of Medicine, Washington University School of Medicine, St Louis, MO 63110.

Vaccine (ENGLAND) 1993, 11 (7) p773-6, ISSN 0264-410X
Journal Code: X60
Contract/Grant No.: 5T32AI07172, AI, NIAID; AI30084, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9311
Subfile: INDEX MEDICUS

The expression of a major surface antigen of the intestinal protozoal parasite Entamoeba histolytica in an attenuated Salmonella typhimurium vaccine strain is described. A polymerase chain reaction fragment derived from cDNA encoding the serine-rich Entamoeba histolytica protein, SREHP, was introduced into S. typhimurium chi 3987 (delta cya delta crp delta **asd**) using a plasmid expression vector (pYA292) containing the aspartate semialdehyde (**asd**) gene. S. typhimurium expressing recombinant SREHP as a SREHP/maltose binding protein fusion protein was administered orally to mice and gerbils (an important animal model for E. histolytica infection) and was recovered from splenic tissue in both species. Our study demonstrates the feasibility of expressing recombinant amoebic proteins in attenuated S. typhimurium strains, and shows that vaccine strains of S. typhimurium can successfully infect the gerbil, a widely used model for amoebic liver abscess and intestinal amoebiasis.

Tags: Animal; Female; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Immune responses to Streptococcus sobrinus surface protein antigen A expressed by recombinant Salmonella **typhimurium.**

Doggett TA; Jagusztyn-Krynicka EK; Curtiss R 3d
Department of Biology, Washington University, St. Louis, Missouri 63130.

Infection and immunity (UNITED STATES) May 1993, 61 (5) p1859-66, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: DE-06669, DE, NIDCR; DE-06673, DE, NIDCR; AI-26186, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9307
Subfile: INDEX MEDICUS

In this study, we used a vaccine strain of Salmonella typhimurium to express antigenic determinants of the SpaA antigen of Streptococcus sobrinus, which is involved in the caries-forming process. We cloned either a single repeat (pYA2901) or three tandem repeats (pYA2905) of the 0.48-kb fragment of the spaA gene, which codes for an important component of the SpaA protein, plus a 1.2-kb minor antigenic determinant and measured the resulting immune responses to SpaA in orally immunized BALB/c mice. The single or triple repeat of the spaA gene fragment was inserted into the **Asd** + vector pYA292 and was transformed into the S. typhimurium delta cya delta crp vaccine strain chi 4072 containing delta **asd** in the chromosome. Female BALB/c mice were then orally immunized with two doses of the S. typhimurium containing either of the two SpaA constructs, and the immune responses to the expressed SpaA protein were assessed. Significant serum immunoglobulin G (IgG) anti-SpaA titers were detected in mice immunized with chi 4072(pYA2905) but not chi 4072(pYA2901). Salivary anti-SpaA IgA titers were minimal and were only detected in mice immunized with S. typhimurium expressing the SpaA encoded by pYA2905. Intestinal anti-SpaA IgA titers, however, were detected in both groups of mice, particularly in mice immunized with chi 4072(pYA2905). An oral booster 26 weeks after the initial series of immunizations resulted in increased serum IgG titers in both chi 4072(pYA2901)- and chi 4072(pYA2905)-immunized animals, particularly in the chi 4072(pYA2905)-immunized animals. No anamnestic IgA response was detected in the saliva following the booster immunization.

Tags: Animal; Female; Support, Non-U.S. Gov't; Support, U.S. Gov't,

Escherichia coli heat-labile toxin subunit B fusions with Streptococcus sobrinus antigens expressed by Salmonella **typhimurium oral vaccine strains: importance of the linker for antigenicity and biological activities of the hybrid proteins.**

Jagusztyn-Krynicka EK; Clark-Curtiss JE; Curtiss R 3d
Department of Biology, Washington University, St. Louis, Missouri 63130.

Infection and immunity (UNITED STATES) Mar 1993, 61 (3) p1004-15, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: AI26186, AI, NIAID; AI23470, AI, NIAID; DE06673; +
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9305
Subfile: INDEX MEDICUS

A set of vectors possessing the genes for aspartate semialdehyde dehydrogenase (**asd**) and the B subunit of the heat-labile enterotoxin of Escherichia coli (LT-B) has been developed. These vectors allow operon or gene fusions of foreign gene epitopes at the C-terminal end of LT-B. Two groups of vectors have been constructed with and without leader sequences to facilitate placing of the foreign antigen in different cell compartments. Two Streptococcus sobrinus genes coding for principal colonization factors, surface protein antigen A (SpaA), and dextranase (Dex), have been fused into the 3' end of the LT-B gene. Resulting protein fusions of approximately 120 to 130 kDa are extremely well recognized by antibodies directed against both SpaA and Dex as well as against LT-B domains and retain the enzymatic activity of dextranase and the biological activity of LT-B in that they bind to GM1 gangliosides. Maximum antigenicity was obtained with the vector possessing an intervening linker of at least six amino acids with two proline residues. Some of the fusion proteins also exhibited another property of LT-B in that they were exported into the periplasm where they oligomerized. LT-B-SpaA and LT-B-Dex hybrid proteins are expressed stably and at a high level in avirulent Salmonella typhimurium vaccine strains which are being used to investigate their immunogenicity and types of induced immune responses. The fusion vectors will also be useful for production and purification of LT-B fusion antigens to be used and evaluated in other vaccine compositions.

Tags: Support, U.S. Gov't, P.H.S.

Vibrio cholerae toxin B subunit gene expressed in a Salmonella **vaccine strain]**

Tian J; Lu D

Second Military Medical University, Shanghai.

[Wei sheng wu hsueh pao] (CHINA) Oct 1992, 32 (5) p320-7, ISSN 0001-6209 Journal Code: XNA

Languages: CHINESE Summary Languages: ENGLISH

Document type: JOURNAL ARTICLE English Abstract

JOURNAL ANNOUNCEMENT: 9304

Subfile: INDEX MEDICUS

This paper reports that the V. cholerae toxin B subunit (ctx B) gene was inserted into pYA 248 plasmid with the aspartate beta-semialdehyde dehydrogenase (**asd**)gene and the recombinant plasmid was transformed into S. typhimurium deleting **asd** gene. Results showed that ctx B gene was highly expressed and secreted into midium. This strain was able to colonize in the intestinal epithelium. Oral immunity and general immunity could produce antibodies at high level and enhance cellular immune responses. The animals orally inoculated with S. typhimurium x 4072 (pYA-ctx B) vaccine had remarkable protection against virulent V. cholear 569B strain and S. typhimurium strain. Use of such system provides useful method for oral vaccine.

Tags: Animal; Female; Male; Support, Non-U.S. Gov't

Cloning and sequencing of a gene encoding a 21-kilodalton outer membrane protein from Bordetella avium and expression of the gene in Salmonella **typhimurium.**

Gentry-Weeks CR; Hultsch AL; Kelly SM; Keith JM; Curtiss R 3d
Laboratory of Microbial Ecology, National Institute of Dental Research, Bethesda, Maryland 20892.

Journal of bacteriology (UNITED STATES) Dec 1992, 174 (23) p7729-42, ISSN 0021-9193 Journal Code: HH3
Contract/Grant No.: 1-F32-AI-07628, AI, NIAID; AI-28487, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9303
Subfile: INDEX MEDICUS

Three gene libraries of Bordetella avium 197 DNA were prepared in Escherichia coli LE392 by using the cosmid vectors pCP13 and pYA2329, a derivative of pCP13 specifying spectinomycin resistance. The cosmid libraries were screened with convalescent-phase anti-B. avium turkey sera and polyclonal rabbit antisera against B. avium 197 outer membrane proteins. One E. coli recombinant clone produced a 56-kDa protein which reacted with convalescent-phase serum from a turkey infected with B. avium 197. In addition, five E. coli recombinant clones were identified which produced B. avium outer membrane proteins with molecular masses of 21, 38, 40, 43, and 48 kDa. At least one of these E. coli clones, which encoded the 21-kDa protein, reacted with both convalescent-phase turkey sera and antibody against B. avium 197 outer membrane proteins. The gene for the 21-kDa outer membrane protein was localized by Tn5seq1 mutagenesis, and the nucleotide sequence was determined by dideoxy sequencing. DNA sequence analysis of the 21-kDa protein revealed an open reading frame of 582 bases that resulted in a predicted protein of 194 amino acids. Comparison of the predicted amino acid sequence of the gene encoding the 21-kDa outer membrane protein with protein sequences in the National Biomedical Research Foundation protein sequence data base indicated significant homology to the OmpA proteins of Shigella dysenteriae, Enterobacter aerogenes, E. coli, and Salmonella typhimurium and to Neisseria gonorrhoeae outer membrane protein III, Haemophilus influenzae protein P6, and Pseudomonas aeruginosa porin protein F. The gene (ompA) encoding the B. avium 21-kDa protein hybridized with 4.1-kb DNA fragments from EcoRI-digested, chromosomal DNA of Bordetella pertussis and Bordetella bronchiseptica and with 6.0- and 3.2-kb DNA fragments from EcoRI-digested, chromosomal DNA of B. avium and B. avium-like DNA, respectively. A 6.75-kb DNA fragment encoding the B. avium 21-kDa protein was subcloned into the **Asd** + vector pYA292, and the construct was introduced into the avirulent delta cya delta crp delta **asd** S. typhimurium chi 3987 for oral immunization of birds. The gene encoding the 21-kDa protein was expressed equivalently in B. avium 197, delta **asd** E. coli chi 6097, and S. typhimurium chi 3987 and was localized primarily in the cytoplasmic membrane and outer membrane.(ABSTRACT TRUNCATED AT 400 WORDS)

Tags: Animal; Comparative Study; Support, U.S.

Cloning and characterization of the asd **gene of** Salmonella **typhimurium: use in stable maintenance of recombinant plasmids in** Salmonella **vaccine strains.**

Galan JE; Nakayama K; Curtiss R 3d
Department of Biology, Washington University, Saint Louis, MO 63130.
Gene (NETHERLANDS) Sep 28 1990, 94 (1) p29-35, ISSN 0378-1119
Journal Code: FOP
Contract/Grant No.: AI24533, AI, NIAID; DE06669
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9102
Subfile: INDEX MEDICUS

Cloning and characterization of the asd **gene of** Salmonella **typhimurium: use in stable maintenance of recombinant plasmids in** Salmonella **vaccine strains.**

Galan JE; Nakayama K; Curtiss R 3d
Department of Biology, Washington University, Saint Louis, MO 63130.

Gene (NETHERLANDS) Sep 28 1990, 94 (1) p29-35, ISSN 0378-1119
Journal Code: FOP
Contract/Grant No.: AI24533, AI, NIAID; DE06669
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9102
Subfile: INDEX MEDICUS

The **asd** mutants of Salmonella typhimurium have an obligate requirement for diaminopimelic acid (DAP) and will undergo lysis in environments deprived of DAP. This has allowed the development of a balanced-lethal system for the expression of heterologous antigens in vaccine strains using vectors containing the wild-type **asd** gene from Streptococcus mutans and **asd** mutant Salmonella hosts [Nakayama et al., Biotechnology 6 (1988) 693-697]. We have cloned the **asd** gene from S. typhimurium, characterized the gene product and used this gene to construct **Asd** + expression cloning vectors. In addition we have constructed an **asd** cassette and a transposon derived from Tn5 that allow the rapid modification of other vectors for use with delta **asd** vaccine strains of S. typhimurium adding versatility to the **Asd** + vector/delta **asd** host system of plasmid maintenance.

Tags: Support, U.S. Gov't, P.H.S.

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1/3/1 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00445018
RECOMBINANT BACTERIAL SYSTEM WITH ENVIRONMENTALLY LIMITED VIABILITY
SYSTEME BACTERIEN RECOMBINE A VIABILITE LIMITEE PAR L'ENVIRONNEMENT
Patent Applicant/Assignee:
  WASHINGTON UNIVERSITY
Inventor(s):
  CURTISS Roy III
  TINGE Steven A
Patent and Priority Information (Country, Number, Date):
  Patent: WO 9640947 A1 19961219
  Application: WO 96US9774 19960607 (PCT/WO US9609774)
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Publication Language: English
Fulltext Word Count: 25180

1/3/2 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0207422 DBA Accession No.: 97-02543 PATENT
Microbial cell containing environmentally limited viability system including essential or lethal gene- biological containment and attenuation of Escherichia coli or a Salmonella sp. for use as a recombinant vaccine with improved safety
AUTHOR: Curtiss III R; Tinge S A
CORPORATE SOURCE: St. Louis, MO, USA.
PATENT ASSIGNEE: Univ.Washington-St.Louis 1996
PATENT NUMBER: WO 9640947 PATENT DATE: 961219 WPI ACCESSION NO.: 97-077274 (9707)
PRIORITY APPLIC. NO.: US 473789 APPLIC. DATE: 950607
NATIONAL APPLIC. NO.: WO 96US9774 APPLIC. DATE: 960607
LANGUAGE: English

1/3/3 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

126114183 CA: 126(9)114183n PATENT
Recombinant microbial system with environmentally limited viability and its use as vaccine
  INVENTOR(AUTHOR): Curtiss, Roy, III; Tinge, Steven A.

LOCATION: USA
ASSIGNEE: Washington University
PATENT: PCT International ; WO 9640947 A1 DATE: 19961219
APPLICATION: WO 96US9774 (19960607) *US 473789 (19950607)
PAGES: 98 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/64A; A61K-039/108B; A61K-039/112B; C12N-015/70B; C12N-015/74B; C12N-001/21B; C12N-001/21J; C12R-001/42J; C12R-001/19J DESIGNATED COUNTRIES: AU; CA; CN; HU; IL; JP; MX; NO; NZ; PL; RU; UA DESIGNATED REGIONAL: AT; BE; CH; DE; DK ; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE
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1. Document ID: US 5389529 A

L12: Entry 1 of 4 File: USPT Feb 14, 1995

DOCUMENT-IDENTIFIER: US 5389529 A
TITLE: Modified lam.beta. signal sequence and processes for producing recombinant neurotrophins

ABPL:
Signal sequences based on LamB have been constructed. These signal sequences facilitate both the synthesis and secretion of neurotrophins in E. coli.

BSPR:
6. Example: Production and Recovery of Recombinant hNGF From E. Coli

BSPR:
Mammalian systems provide the most natural environment for the production of mammalian proteins. However, the production of large quantities of proteins in these systems is very expensive. Therefore, there is a need to develop systems that are both less expensive and more productive. One such system is E. coli.

BSPR:
Researchers have attempted to produce neurotrophins in E. coli, but without significant success. Gray and Ullrich (EP 0 121 338, 1984) constructed an expression vector encoding N-methionyl-hNGF and expressed the gene in E. coli. They reported identification of hNGF by immunodetection on Western blot, but they did not isolate the protein or demonstrate biological activity.

BSPR:
Hu and Neet (1988, Gene: 57-65) attempted to express mouse NGF in E. coli. They cloned a DNA sequence encoding a mature mouse NGF in which they replaced serine, the N-terminal amino acid in the native protein, with methionine. They inserted the DNA sequence into a plasmid having a temperature inducible lambda P.sub.L promoter. This system expresses other heterologous proteins at rates of 10%-25% total cellular protein. They expressed the gene and isolated NGF by ammonium sulfate precipitation followed by dialysis against acetate buffer. However, as tested by bioassay, this system yielded only 0.0005% to 0.1% NGF. The authors speculated that the highly inconsistent and low yields were due to toxicity of NGF to the cells, instability or translational inefficiency of the mRNA, or mismatched disulfide bonds in the refolded, oxidized protein.

BSPR:
Iwai et al. (1986, Chem. Pharm. Bull. 34:4724-4730) reported synthesis of a gene encoding hNGF with codons preferred in E. coli. They expressed the gene directly as N-methionyl-hNGF or as a fusion protein with human growth hormone. Direct expression was only one-fourth as efficient as expression of the fusion protein. They examined the proteins by SDS-PAGE, but did not otherwise isolate them.

BSPR:
In summary, previous attempts to express neurotrophins in E. coli have resulted in cell death, low levels of protein accumulation, and expression of protein with virtually no biological activity.

BSPR:
In a further embodiment of the invention, the neurotrophin molecule expressed in E. coli and recovered in a biologically active form is neurotrophin-4. In a

preferred embodiment of the invention directed to expression of biologically active NT-4 in E. coli, the NT-4 is encoded by a nucleic acid molecule comprising a sequence substantially as set forth for hNT-4 in FIGS. 11A-11C (SEQ ID NO: 22) or may comprise a sequence that is at least about seventy percent homologous to such a sequence.

BSPU:
7. Example: Production And Recovery Of Recombinant hBDNF And hBDNFmyc From E. Coli

BSPU:
9. Example: Production And Recovery Of Recombinant hNT-4 from E. Coli

BSPV:
9.2Purification of Human NT-4 Expressed In E. Coli RFJ26/pRG173

DRPR:
FIG. 1 is a schematic representation of plasmid pRPN133. The solid line represents pBR322-derived DNA sequences with the origin of replication (ORI) and the .beta.-lactamase gene (ampicillin resistance) (Ap) indicated. Distinctive features of the plasmid are indicated by boxed regions with arrowheads indicating the direction of transcription or replication. PL indicates the lambda P.sub.L promoter. rbsl is the wild type promoter and ribosome binding site of phage T7 .phi.1.1. The hNGF gene encodes a mature polypeptide in which the second amino acid residue, serine is replaced by threonine. cI857 indicates the heat inactivatable .lambda. repressor gene.

DRPR:
FIGS. 2A-B show dose-response curves to recombinant human NGF produced in E. coli by (FIG. 2A) E8 chick embryo dorsal root ganglia (DRG) explants and (FIG. 2B) dissociated E8 DRG.

DRPR:
FIG. 4 depicts the signal sequence processing kinetics of the modified LamB signal sequences, LamB1 and LamB3. We cultured strain BL21/DE3 containing the appropriate LamB-hBDNF plasmid. In this strain, the gene encoding T7 RNA polymerase has been inserted in the chromosome and is under the transcriptional control of the lac promoter (Studier and Moffatt, J. Mol. Biol. 189:113-30). Addition of IPTG to the growing cells for 10 minutes allows synthesis of T7 RNA polymerase. Subsequent addition of rifampicin to 0.2 mg/ml blocks transcription by E. coli RNA polymerase but allows transcription by T7 RNA polymerase. This results in selective transcription of the LamB--hBDNF gene which is placed immediately downstream from the T7 late .phi.1.1 promoter and ribosome binding site of rbs2. Cells were pulsed with .sup.35 S-methionine for 30 seconds and then chased with an excess of cold methionine. The cultures were sampled at the indicated times after chase and the labelled proteins were analyzed by SDS-15% PAGE and fluorography. Processing was determined by densitometric scanning of the precursor and mature forms of LamB-hBDNF.

DRPR:
FIG. 9 depicts a dose response curve for the stimulation of E8 chicken embryo DRG neurite outgrowth by recombinant hBDNF purified from E. coli. The hBDNF was purified to greater than 95% by C4 reverse phase HPLC.

DRPR:
Plasmid pRG91 (Regeneron Pharmaceuticals) is a pBR322-based vector designated for the expression of recombinant proteins and their secretion into the periplasmic space of Escherichia coli. The vector consists of the strong, regulated, lacUV5 promoter followed by the phage T7 .phi.1.1 promoter and ribosome binding site inserted between the EcoRI and NruI restriction sites in pBR322. These control elements direct the expression of the LamB2 signal sequence to which recombinant protein gene sequences may be fused. The DNA sequences between the unique NruI and PvuII restriction sites were deleted, resulting in increased plasmid copy number. This plasmid confers ampicillin (Ap) resistance.

DRPR:
FIG. 13 depicts the effect of hNT-4 on CAT activity. Treatment of motor neuron enriched cultures with a partially purified extract from an induced culture of strain RFJ26 containing plasmid pRG173 resulted in a 3.6-fold (at 1:20 dilution)

increase in CAT activity after 48 hours as compared to untreated (C-NT) and buffer (C-buffer) controls. The E. coli extract was passed through a Sepharose-S column as disclosed infra prior to treatment of motor neuron enriched cultures.

DEPR:
In a specific embodiment of the invention, sequences encoding human NT-4 are expressed in an E. coli expression system and a purification scheme as disclosed infra is used to produce useful amounts of human NT-4. The nucleic acid encoding human NT-4 which is thus expressed can be that contained in nucleic acid pRG173 (ATCC Accession Number 75131) or HG7-2 (ATCC Accession Number 75070) or shown in FIGS. 11A-C (SEQ ID NO: 22), or isolated by any methods known in the art, or as follows: Mixtures of 5' and 3' oligonucleotides representing all possible codons corresponding to known NT-4 sequences or to conserved amino acid sequences from known neurotrophins are utilized as primers in the polymerase chain reaction (PCR). Primary and secondary PCR amplification reactions of human (or other mammalian) cDNA or genomic libraries result in the isolation of a PCR product that can be utilized as .sup.32 P-labelled probes to isolate a full length cDNA or genomic clone encoding NT-4. The term "human neurotrophin-4" as used herein should be understood as meaning any human homologue of the Xenopus NT-4 (Hallbook, et al., 1991, Neuron 6:845-858), including a distinct yet homologous (e.g., at least about seventy percent homology) neurotrophin molecule.

DEPR:
The literature discloses a variety of expression control sequences useful for expressing DNA sequences in transformed non-animal hosts. These include, among others, in bacteria, the lac system, the trp system, the TAC system, the TRC system, the lambda P.sub.L promoter, the T7 late promoters, and the control regions of the fd coat protein; and in yeast, the phosphoglycerate kinase promoter, the Gal 4 promoter, the His promoter, the alcohol dehydrogenase promoter, the alkaline phosphatase promoter and the .alpha.-mating factor promoter. Controllable expression control sequences are preferable and, among these, a temperature inducible lambda P.sub.L promoter, the lacUV5 promoter and the T7 .phi.1.1 promoter are most preferable for expression in E. coli.

DEPR:
The art is also familiar with many non-animal hosts useful to express heterologous proteins, including E. coli, Bacillus, Streptomyces, Saccharomyces and Pichia pastoris. We prefer E. coli.

DEPR:
The expression of the heat shock genes is regulated by heat shock regulatory genes, such as the htpR gene of E. coli. Mutants of the HtpR gene are deficient in expression of heat shock protease genes, as well as other genes that contribute to the heat shock response. We have found that expressing recombinant neurotrophins in heat shock regulatory gene mutants, especially htpR.sup.- mutants, significantly improves yield. HtpR.sup.- lon.sup.- double mutants are particularly useful. We prefer the E. coli strain LC137, an htpR.sup.ts lon.sup.- mutant. This strain is available from Prof. Alfred Goldberg, Harvard University. U.S. Pat. No. 4,758,512 (Goldberg et al.) describes other suitable strains. Another preferred E. coli strain is RFJ26.

DEPR:
The inability of E. coli to accumulate neurotrophins may be the result of one or more properties of the neurotrophin gene or protein. The structure of the neurotrophin mRNA, particularly the structure proximal to the translation start point, may prevent efficient translation. Alternatively, the neurotrophin may prevent its own synthesis by interacting directly with its mRNA, or it may interact directly or indirectly with some component of the DNA replication/transcription/translation machinery of E. coli.

DEPR:
According to another embodiment of our invention, we secrete the neurotrophin into the periplasmic space of E. coli rather than express it intracellularly. We accomplish this by fusing a signal sequence to a mature neurotrophin. A signal sequence gene fused to the 5' end of the neurotrophin gene may provide a nucleotide sequence proximal to the translation startpoint that is more conducive to efficient translation, thus resulting in higher levels of neurotrophin accumulation. In addition, sequestering the neurotrophin in the periplasmic space prevents it from interfering with any cytosolic component necessary for protein

synthesis. It also protects it from attack by cytosolic proteases. It is also possible that the secretion of a mature neurotrophin into the periplasmic space may provide an environment more conducive to the proper folding of the protein.

DEPR:
A signal sequence is provided by constructing a recombinant DNA molecule in which the DNA sequence encoding the neurotrophin comprises, from 5' to 3' a fused gene encoding a signal or leader sequence appropriate to the host cell which is in-frame with a DNA sequence for the neurotrophin. The literature describes several signal sequences useful in such constructions. For example, LamB, OmpA and PhoA are useful in E. coli. (Denefle et al., 1985, Gene 85:499-510; Wong et al., 1988, Gene 68:193-203). We prefer LamB and, in particular, modified LamB signal sequences that improve the translational efficiency of the LamB mRNA. We constructed genes for modified LamB signal sequences in the following manner. We made degenerate substitutions to the third nucleotide of several codons of LamB, replacing G or C with A or T. These substitutions do not change the amino acid sequence of the LamB signal peptide, but do decrease the potential number of hydrogen bonds in any secondary structure. This reduces the stability of possible secondary structures involving this region of LamB mRNA. We also introduced codon changes based on codon usage models, to more nearly approximate codons used most frequently by E. coli.

DEPR:
We also modified the LamB signal sequence to improve efficiency of processing of the Lamb precursor protein into mature protein. Native LamB has a hydrophobic core of 10 amino acid residues. Mutational analysis of several E. coli signal sequences suggests that the length of the hydrophobic core region can have a strong effect on signal sequence activity. We have found that increasing the length of the hydrophobic region by the addition of up to ten hydrophobic amino acid residues improves the efficiency of processing LamB fusion precursor polypeptides. Fewer than six is preferable and four is most preferable. The choice of hydrophobic amino acids added is not critical, nor is the precise location at which they are added to the hydrophobic core region. However, we prefer to add the tetra-peptide Leu-Ala-Val-Leu ("LAVL") (SEQ ID NO: 6) at a convenient restriction site near the N-terminal end of the hydrophobic region. We describe particular genes for modified Lamb signal sequences in Example 7.

DEPR:
Native neurotrophins are soluble in neutral buffers. However, recombinant neurotrophins from E. coli behave as insoluble proteins. Recombinant neurotrophins have been detected in the cytosolic fraction and, when exported, have been recovered from the periplasmic space using standard techniques such as osmotic shock, spheroplasting, or freeze-thaw (Bochner et al., U.S Pat. No. 4,680,262). However, they are isolable only as a small fraction of the total neurotrophins in the cell.

DEPR:
As described in an example section infra, the present invention discloses the expression of biologically active human neurotrophin-4. The human NT-4 DNA sequence was subcloned into the DNA plasmid vector pRG91, resulting in pRG173. This hNT-4 containing plasmid was transformed into E. coli strain RFJ26, and methods described in the instant specification were utilized to recover biologically active NT-4 from the culture system. However, applicants are not to be limited to such a specific embodiment. For example, any nucleic acid sequence substantially homologous to the region of HG7-2 encoding human NT-4 can be utilized to construct any number of DNA plasmid expression vectors as described throughout the specification or known to the skilled artisan, which in turn can be utilized to transform any number of E. coli bacterial strains in order to produce useful amounts of biologically active NT-4.

DEPR:
6. EXAMPLE: PRODUCTION AND RECOVERY OF RECOMBINANT hNGF FROM E. COLI

DEPR:
We transformed the E. coli htpR.sup.ts Rts lon.sup.- mutant strain, LC137, with RPN133 to yield pRPN133/LC137.

DEPR:
The biological activity of the purified protein was tested for neurite outgrowth

in E8 explanted and dissociated dorsal root ganglia (FIG. 2A and 2B) (Lindsay et al., 1985, Dev. Biol. 112:319-328). By this criteria the recombinant hNGF purified from E. coli by this method was found to be as active as NGF purified from mouse salivary gland.

DEPR:
7. EXAMPLE: PRODUCTION AND RECOVERY OF RECOMBINANT hBDNF and hBDNFmyc FROM E. COLI

DEPR:
We constructed signal sequences based on LamB to facilitate both the synthesis and secretion of neurotrophins in E. coli. The Lamb signal sequence (FIG. 3A, SEQ ID NO: 1) is a naturally occurring E. coli signal sequence which was selected for the construction of a series of secretion vectors based on the pRPN series of expression vectors developed at Regeneron Pharmaceuticals, Inc. Secretion vectors were constructed by cloning synthetic DNA fragments encoding the Lamb signal sequence into pRPNO9 or pRPN16. These plasmids derive from expression vector pNKS97 (Panayotatos, 1987, Engineering an Efficient Expression System, In: Plasmids-A Practical Approach, ed. Herdy, K., IRL Press, Oxford/Washington, D.C.) into which have been inserted a lacUV5 promoter. LamB was inserted into the structural gene insert site such that expression of Lamb is under control of the lacUV5 or T7 .phi.1.1 promoter.

DEPR:
The fusion of mature hBDNF to LamB1 results in efficient synthesis of the fusion protein in E. coli. The authenticity of the synthesized product was confirmed by selective synthesis in a DNA-dependent coupled transcription-translation cell-free protein synthesis system, by the selective synthesis of the product using a T7 RNA polymerase expression system in E. coli, and by the synthesis of the product with a C-terminal myc tag allowing for identification of the chimera with a myc-specific monoclonal antibody. Either one of these fusion proteins synthesized in E. coli was processed to mature hBDNF as evidenced by its mobility on SDS-PAGE. The level of expression obtained with LamB1 or LamB2 results in accumulation of hBDNF from about 1% to 10% of total cell protein.

DEPR:
We transformed E. coli W3110 I.sup.q F.sup.- with pRPN121 to produce W3110 I.sup.q F.sup.- /pRPN121.

DEPR:
We transformed E. coli I.sup.q FW3110 with pRPN149. Then we produced and recovered recombinant hBDNF by the procedure described in Example 7.

DEPR:
This DNA fragment has a C-terminal EagI restriction site suitable for insertion into BalI and EagI sites of pRPN88, where it would replace the hBDNF DNA sequence. The resulting plasmid is used to transform E. coli I.sup.q FW3110. Then recombinant hNT-3 is produced and recovered as in Example 7. We expect recombinant hNT-3 purified from E-coli to exhibit neurotrophin activity similar to that described by Hohn et al., 1991, (WO 91/03569).

DEPR:
9. EXAMPLE: PRODUCTION AND RECOVERY OF RECOMBINANT hNT-4 FROM E. COLI

DEPR:
9.2. PURIFICATION OF HUMAN NT-4 EXPRESSED IN E. COLI RFJ26/pRG173

DEPR:
A 5 ml culture of pRG173 transformed in E. coli strain RFJ26 was grown overnight in LB medium, supplemented with 100 mg/L ampicillin at 37.degree. C. with aeration. The overnight culture was diluted into 500 ml LB and grown to OD.sub.590 =5.3 at which time the expression of hNT-4 was induced by the addition of lactose to 1% (w/v) and the culture was grown overnight with aeration. Cells were then collected by centrifugation and frozen at 70.degree. C. The cell pellet (7.2 grams) was thawed and resuspended in 73 ml of 200 mM Tris-HCl, pH 8.0, 50 mM EDTA and lysed by 3 sequential passes through the STANSTED.RTM. cell disrupter. The lysate was then centrifuged at 26,000.times.g for 10 minutes, yielding 83 ml of supernatant containing the soluble fraction. The soluble fraction was dialyzed against 50 mM Tris-HCl, pH 8.5 at 4.degree. C. for 5 hrs then diluted 10-fold

with 20 mM MES, pH 6.0 and loaded on a Fast S-Sepharose column equilibrated with 20 mM MES, pH 6.0 and eluted with 1M NaCl in 20 mM MES, pH 6.0. Recombinant human NT-4 protein that stimulated E8 DRG outgrowth was recovered in the 1M NaCl wash.

DEPR:
Subcloning the NT-4 coding region of bacteriophage HG7-2 downstream from the LAC UV5 and T7.phi.1.1 promoters, the T7.phi.1.1 ribosome binding site and the LamB signal sequence resulted in plasmid pRG173. Transformation of pRG173 into the E. coli strain RFJ26 and induction of large scale cultures of pRG173/RFJ26 resulted in the expression of a biologically active form of recombinant human NT-4. The ability to express a biologically active form of human NT-4 in an recombinant prokaryotic expression system substantially increases the ease at which the production of human recombinant NT-4, peptides or derivatives thereof may be scaled up for both therapeutic and diagnostic applications.

CLPR:
5. A process for producing a recombinant neurotrophin comprising the steps of culturing E. coli host cells transformed with a recombinant DNA molecule comprising an expression control sequence operatively linked to a fused gene consisting of, from 5' to 3' the LamB signal sequence gene of claim 1 or claim 4 joined in-frame with a neurotrophin encoding sequence, under conditions such that said neurotrophin is expressed by said E. coli host cells; and recovering the expressed neurotrophin.

CLPR:
6. The process of claim 5 wherein the neurotrophin is human neurotrophin-4, wherein said human neurotrophin-4 is encoded by a DNA sequence as contained in E. coli plasmid vector, pRG173, as deposited with the ATCC and assigned accession number 75131.

ORPL:
Stader et al., 1990, Methods In Enzymology, vol. 185 "Engineering Escherichia coli to Secrete Heterologous Gene Products", pp. 166-187.

ORPL:
Goff, S. A., et al., 1984, "Heat Shock Regulatory Gene htpR Influences Rates of Protein Degradation and Expression of the Ion Gene in Escherichia coli," Proc. Natl. Acad. Sci. USA, 81:6647-51.

ORPL:
Hedgpeth, J., et al., 1980, "DNA Sequence Encoding the NH.sub.2 Terminal Peptide Involved in Transport of .lambda. Receptor, an Escherichia coli Secretory Protein," Proc. Natl. Acad. Sci. USA, 77:2621-2625.

ORPL:
Hu, G., and K. E. Neet, 1988, "Expression of the cDNA for Mouse .beta.-Nerve Growth Factor Protein in Escherichia coli,"Gene, 70:57-65.

ORPL:
Kawaguchi, Y., et al., 1984, "Renaturation and Activation of Calf Prochymosin Produced in an Insoluble Form in Escherichia coli," J. of Biotechnology, 1:307-315.

ORPL:
Marston, F. A. O., et al., 1984, "Purification of Calf Prochymosin (Prorennin) Synthesized in Escherichia coli," Bio/Technology, Sep. 1984, pp. 800-804.

ORPL:
Miller, K. W., et al., 1989, "Secretory Leukocyte Protease Inhibitor Binding to mRNA and DNA as a Possible Cause of Tosicity to Escherichia coli," J. Bacteriol., 171(4):2166-72.

ORPL:
Obukowicz, M. G., et al., 1988, "Secretion and Export of IGF-1 in Escherichia coli Strain JM101," Mol. Gen. Genet., 215:19-25.

ORPL:
Wong, E. Y., et al., 1988, "Expression of Secreted Insulin-like Growth Factor-1 in Escherichia coli," Gene, 68:193-203.

DEPC:
EXAMPLE 4: EXAMPLE ADDED TO NKAF PATENT APPLICATION (NKAF expression in Escherichia coli)

DEPC:
2. NKAF expression in E. coli

DETL:
TABLE 13 ______________________________________ NKAF expression in E. coli N 4840 Time after teperature shift Plasmid 0 1.5 3 5 7.5 h ______________________________________ pNK8001 3 5 26 33 19 ng/ml pPL9-5001 2 -- -- -- 3 ______________________________________

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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3. Document ID: US 5304473 A

L12: Entry 3 of 4 File: USPT Apr 19, 1994

DOCUMENT-IDENTIFIER: US 5304473 A
TITLE: A-C-B proinsulin, method of manufacturing and using same, and intermediates in insulin production

BSPR:
The action of methionyl amino peptidase (MAP), a protein indigenous to E. coli, will remove an N-terminal deformylated methionine provided the second residue is not arginine, aspartate, glutamine, glutamate, isoleucine, leucine, lysine or methionine. Examination of the primary structure of the insulin molecule, human insulin being a representative example shown in FIG. 29, demonstrates that the-N-terminal residue of the B-chain, corresponding to the N-terminal residue of natural proinsulin, is phenyalanine. This transcriptional and translational order prevents the removal of the N-terminal methionine of the recombinantly E. coli produced proinsulin molecule by MAP. However, the N-terminal amino acid of the A-chain is glycine whose presence does not inhibit the action of MAP. Thus, if one could reverse the sequence of translation from B-chain/C-peptide/A-chain to A-chain/C-peptide/B-chain, the intrinsic action of MAP would eliminate the N-terminal methionine. This would consequently obviate the need for post-translational removal of the N-terminal Met thereby incurring a substantial commercial and technical advantage.

DEPR:
Approximately 5 .mu.l of the vector DNA was mixed with 10 picomoles of the synthetic DNA fragments, corresponding to the two halves of the ACB-PI synthetic gene, in 50 .mu.l of ligation buffer (50 mM tris-HCl, pH 7.6, 100 mM MgCl2, 10 mM DTT (dithiothreitol), 800 mM ATP, and 3.5 units of T4 DNA ligase (commercially available from BoehringerMannheim Biochemicals, Indianapolis, Ind. 46250). The reaction was then incubated at 4.degree. C. overnight and then transformed into frozen competent E. coli DH5.alpha. cells (commercially available from Bethesda Research Laboratories, Inc., P. O. Box 6009, Gaithersburg, Md. 20877) by techniques well known in the art and deliniated in standard laboratory manuals such as Sambrook, J., at al., supra. The transformants of the preferred embodiment of the invention were grown at 37.degree. C. overnight x-gal TY agar plates containing 100 .mu.g/ml ampicillin. The choice of antibiotic and media is dependent on the amplification vector and cell line employed.

DEPR:
The expression plasmid to be used, which may be selected from a number of alternatives, possesses an appropriate control region and appropriate restriction sites facilitating integration of the ACE-PI coding sequence operably with respect to the control regions. A variety of expression vectors useful for transforming procaryotic and transfecting eucaryotic cells are well known in the art. Examples of said expression vectors include pTrc 99A, pKK223-3, pKK223-2,

pDR540 tac promoter vector, pDR trp promoter vector, pcz20, pLEBBGH2, and pL110C. In the most preferred practice of the invention as exemplified herein when the host cell is an E. coli K12 cell, the expression vector was pCZR126S. This plasmid may be prepared according to the teaching of Example 3 herein.

DEPR:
So as to achieve efficient transcription of the synthetic gene, said gene must be operably associated with a promoter operator region. A variety of promoter-operator regions functional in E. coli host cells are well known in the art. In the preferred practice of the invention as exemplified herein, said promoter-operator region is the lambda pL promoter operator region.

DEPR:
As exemplified herein, the ligation mixture was diluted with 50 .mu.l of 10 mM tris-HCl (pH 7.6) and 3 .mu.l of CaCl.sub.2 and subsequently used to directly tranforn competent E. coli K12 RV308 cells as provided in Example 3A herein. In the preferred embodiment of the invention E. coli K12 RV308 cells were employed as host cells but numerous other cell lines are available such as, but not limited to, E. coli K12 L201, L687, L693, L507, L640, L641, L695, L814 (E. coli B). The transformed host cells are then plated on appropriate media under the selective pressure of the antibiotic corresponding to the resistance gene present on the expression plasmid. The cultures are then incubated for a time and temperature appropriate to the host cell line employed.

DEPR:
The techniques of transforming cells with the aformentioned vectors are well known in the art and may be found in such general references as Maniatis, et al. (1988) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York or Current Protocols in Molecular Biology (1989) and supplements. The methodology for transforming the E. coli cell lines employed in the most preferred practice of the invention may be obtained by reference to the Examples section herein. The precise conditions under which the transformed E. coli cells are cultured is dependent on the nature of the E. coli host cell line and the expression or cloning vectors employed. For example, vectors which incorporate thermoinducible promoter-operator regions, such as the cl857 thermoinducible lambda-phage promoter-operator region, require a temperature shift in the culture conditions so as to induce protein synthesis.

DEPR:
2. incorporating said gene into a suitable vector containing a promoter-operator region functional in an E. coli host cell,

DEPR:
4. transforming an E. coli host cell with said vector,

DEPR:
5. culturing said transformed E. coli host cell under conditions appropriate so as to induce transcription and translation of said gene,

DEPR:
This novel pathway for the preparation of insulin is distinct from the current practice of replicating natural processes in diverse organisms. This alternate pathway to insulin results in significant savings in the recombinant production of commercially significant quantities of insulin by eliminating the requirement of removing the N-terminal methionine of the recombinant molecule with cathepsin C, or other methods, relying instead on the intrinsic action of the methionyl amino peptidase of the E. coli host cell to remove the N-terminal methionine.

DEPR:
Since the removal of the N-terminal methionine residue of ACB-PI is dependent on the presence of MAP, the host cell chosen must intrisically produce MAP or have been engineered to produce MAP. The MAP protease is indigenous to E. coli cells. Thus, a variety of E. coli cell lines which are not deficient in the production of the MAP may be employed in the practice of the method of the instant invention. Examples of E. coli host cells useful in the practice of the instant invention include the cell lines E. coli K12 L201, L687, L693, L507, L640, L641, L695, L814 (E. coli B). In the pre erred practice of the invention said E. coli host cell is the E. coli K12 RV308 E. coli cell line.

DEPR:
About 5 .mu.l of this vector DNA was mixed with 10 picomoles of the two synthetic DNA fragments as prepared above in 50 .mu.l of ligation buffer (50 mM tris-HCl, 10 mM MgCl.sub.2, 10 mM DTT, 800 uM ATP, and 3.5 units of T4 DNA ligase, pH=7.6). The reaction was incubated at 4.degree. C. overnight and then transformed into frozen competent E. coli DH5 cells (commercially available from Bethesda Research Laboratories, P. O. Box 6009, Gaithersburg, Md. 20877). The transformants were grown at 37.degree. C. overnight on x-gal TY agar plates containing 100 .mu.g/ml of ampicillin. Clones containing the correct insert were chosen by the loss of a functional lacZ gene as screened by the blue/white colony selection and confirmed with ds-DNA sequencing using the Sequenase kit (commercially available from United States Biochemical Corp.). The resulting plasmid was designated pRB181.

DEPR:
Lyophils of E. coli K12 BE1201/pKC283 are obtained from the Northern Regional Research Laboratory, Peoria, Ill. 61604, under the accession number NRRL B-15830. The lyophils are decanted into tubes containing 10 ml LB medium (10 g Bacto-tryptone, 5 g Bacto-yeast extract, and 10 g NaCl per liter; pH is adjusted to 7.5) and incubated two hours at 32.degree. C., at which time the cultures are made 50 .mu.g/ml in ampicillin and then incubated at 32.degree. C. overnight. The E. coli K12 BE1201/pKC283 cells were cultured at 32.degree. C., because the cells comprise a temperature-sensitive cI repressor gene integrated into the cellular DNA. When cells that comprise a wild-type lambda pL repressor gene or do not comprise a lambda pL promoter are utilized in this plasmid isolation procedure, as described in subsequent Examples herein, the temperature of incubation is 37.degree. C.

DEPR:
A small portion of the overnight culture is placed on LB-agar (LB medium with 15 g/l Bacto-agar) plates containing 50 mg/ml ampicillin in a manner so as to obtain a single colony isolate of E. coli K12 BE1201/pKC283. The single colony obtained was inoculated into 10 ml of LB medium containing 50 .mu.g/ml ampicillin and incubated overnight at 32.degree. C. with vigorous shaking. The 10 ml overnight culture was inoculated into 500 ml LB medium containing 50 .mu.g/ml ampicillin and incubated at 32.degree. C. with vigorous shaking until the culture reached stationary phase.

DEPR:
E. coli K12 Mo (.lambda..sup.+) can be obtained brom the Northern Regional Research Laboratories in lyophylized form under the accession number NRRL B-15993. E. coli K12 MO(.lambda..sup.+) comprises the wildtype lambda pL cI repressor gene, so that transcription from the hybrid pL-1pp promoter of the present invention does not occur in E. coli K12 MO(.lambda..sup.+) cells. The lyophils are reconstituted, single colonies of MO (.lambda..sup.+) are isolated, and a 10 ml overnight culture of the MO(.lambda..sup.+) cells is prepared in substantial accordance with the procedure of Example 29A1, except that the temperature of incubation is 37.degree. C. and no ampicillin is used in the growth media.

DEPR:
The colonies were individually cultured, and the plasmid DNA of the individual colonies was examined by restriction enzyme analysis and gel electrophoresis. Plasmid DNA isolation was performed on a smaller scale in accordance with the procedure of Example 29A1, but the CsCl gradient step was omitted until the desired E. coli K12 MO.lambda..sup.+)/pKC283PX transformants were identified. A restriction site and function map of plasmid pKC283PX is presented in FIG. 2 of the accompanying drawings.

DEPR:
The linker and BglII-XhoI-digested plasmid pKC283PX were ligated in substantial accordance with the procedure of Example 3A2. The ligated DNA constituted the desired plasmid pKC283-L. A restriction site and function map of plasmid pKC283-L is presented in FIG. 3 of the accompanying drawings. The plasmid pKC283-L DNA was used to transform E. coli K12 MO(.lambda..sup.+) and the resulting E. coli K12 MO(.lambda..sup.+)/pKC283-L transformants were identified in substantial accordance with the procedure of Example 3A3.

DEPR:
About 10 .mu.g of plasmid pKC283-L DNA, prepared in substantial accordance with

the procedures of Example 29A1, were dissolved in 20 .mu.l 10X high-salt buffer, 20 .mu.l 1 mg/ml BSA, 5 .mu.l (.about.50 units) restriction enzyme XhoI, and 155 .mu.l of H.sub.2 O, and the resulting reaction was incubated at 37.degree. C. for two hours. The XhoI-digested plasmid pKC283-L DNA was then precipitated from the reaction mixture by the addition of three volumes of 95% ethanol and one-tenth volume of 3M sodium acetate, incubated in a dry ice-ethanol bath for five minutes, and centrifugation. The resulting DNA pellet was washed with 70% ethanol, dried, and resuspended in 2 .mu.l 10X nick-translation buffer (0.5M Tris-HCl, pH 7.2; 0.1M MgSO.sub.4 ; and 1 mM DTT), 1 .mu.l of a solution 2 mM in each of the deoxynucleotide triphosphates, 15 ml of H.sub.2 O, 1 ml (.about.6 units as defined by P-L Biochemicals) of Klenow, which is the large fragment of E. coli DNA polymerase I, and 1 .mu.l of 1 mg/ml BSA. The resulting reaction was incubated at 25.degree. C. for 30 minutes; the reaction was stopped by incubating the solution at 70.degree. C. for five minutes.

DEPR:
The .about.5.9 kb BamHI restriction fragment was circularized by ligation and transformed into E. coli K12 MO(.lambda..sup.+) in substantial accordance with the procedures of Examples 3A2 and 3A3. The E. coli K12 MO(.lambda..sup.+)/pKC283-LB transformants were identified, and then plasmid pKC283-LB DNA was prepared in substantial accordance with the procedure of Example 3A1. A restriction site and function map of plasmid pKC283-LB is presented in FIG. 4 of the accompanying drawings.

DEPR:
About 10 mg of plasmid pKC283PX were digested with restriction enzyme SalI in high-salt buffer, treated with Klenow, and ligated to EcoRI linkers (5'-GAGGAATTCCTC-3')(Seq. ID No.8) in substantial accordance with the procedure of Example 3A5, with the exception of the starting plasmid, restriction enzymes, and linkers used. After digestion with restriction enzyme EcoRI, which results in the excision of .about.2.1 kb of DNA, the .about.4.0 kb EcoRI restriction fragment was circularized by ligation to yield plasmid pKC283PRS. The ligated DNA was used to transform E. coli K12 MO (.lambda..sup.+) in substantial accordance with the procedure of Example 3A3. After the E. coli K12 MO(.lambda..sup.+)/pKC283PRS transformants were identified, plasmid pKC283PRS DNA was prepared in substantial accordance with the procedure of Example 3A1. A restriction site and function map of plasmid pKC283PRS is presented in FIG. 5 of the accompanying drawings.

DEPR:
The .about.0.85 kb PstI-SphI restriction fragment of plasmid pKC283PRS was ligated to the .about.3.0 kb PstI-SphI restriction fragment of plasmid pKC283-LB in substantial accordance with the procedure of Example 3A2. The ligated DNA constituted the desired plasmid pL32. A restriction site and function map of plasmid pL32 is presented in FIG. 6 of the accompanying drawings. Plasmid pL32 was transformed into E. coli K12 MO(.lambda..sup.+) cells in substantial accordance with the procedure of Example 3A3. Plasmid pL32 DNA was prepared f rom the E. coli K12 MO (.lambda..sup.+) /pL32 transformants in substantial accordance with the procedure of Example 3A1. Analysis of the plasmid pL32 DNA demonstrated that more than one EcoRI linker attached to the Klenow-treated, SalI ends of plasmid pKC283PX. The presence of more than one EcoRI linker does not affect the utility of plasmid pL32 or derivatives of plasmid pL32 and can be detected by the presence of an XhoI restriction site, which is generated whenever two of the EcoRI linkers are ligated together. Alternatively, plasmid pL32 may be constructed by carrying out the SalI-EcoRI excision and ligation of the first paragraph of this Example upon plasmid pKC283-LB.

DEPR:
E. coli K12 RV308/pNM789 can be obtained from the Northern Regional Research Laboratories in lyophilized form under the accession number NRRL B-18216. A restriction site and function map of pNM789 is presented in FIG. 7 of the accompanying drawings. Plasmid DNA is extracted from the culture in substantial accordance with the teaching of Example 1, except that the temperature of incubation is 37.degree. C. Ten micrograins of pNM789 are suspended in 200 .mu.l PvuII buffer (50 mM Tris-HCl (pH 7.5), 60 mM NaCl and 6 mM MgCl.sub.2). One unit of PvuII is added and the reaction mix is incubated for 5 minutes at 37.degree. C. The enzyme is inactivated by heating 10 minutes at 65.degree. C. 30 .mu.l of 10X BamHI buffer (200 mM Tris-HCl (pH 8.0), 1M NaCl and 70 mM MgCl.sub.2), 70 .mu.l H.sub.2 O and 10 units of BamHI are next added and the reaction is

incubated for 1 hour at 37.degree. C. This is followed by the addition of 5 units of alkaline phosphatase and incubation for 1 hour at 65.degree. C. The DNA fragments are separated on a 1 percent agarose gel, and a DNA fragment (FIG. 8) the size of a single cut fragment is purified.

DEPR:
A DNA linker with a blunt end and a BamHI end is synthesized in substantial accordance with the teaching of Example 3A4. This linker (shown at 118 in FIG. 8) has the following structure: ##STR9## The linker is kinased and ligated into the BamHI-PvuII digested plasmid pNM789 in substantial accordance with the teaching of Example 3A2. This ligation mixture is used to transform E. coli K12 RV308 cells and plasmid isolation is performed upon these transformants in substantial accordance with the teaching of Example 3A3. Several plasmids are selected which contain the appropriate size PvuII fragment (494bp) and 2WI-BamHI fragment (628bp). The sequence of at least two of these is determined by sequencing from the BamHI site toward the unique SmaI site and one clone is selected with the desired sequence. This intermediate plasmid is designated plasmid 120. A schematic outline of this procedure and a restriction site and function map of plasmid 120 is presented in FIG. 8 of the accompanying drawings.

DEPR:
Plasmid pL32 was also digested with restriction enzymes XbaI and BamHI, and the .about.3.9 kb restriction fragment was isolated and prepared for ligation. The .about.3.9 kb XbaI-BamHI restriction fragment of plasmid pL32 was ligated to the .about.0.6 kb XbaI-BamHI restriction fragment of plasmid 120 in substantial accordance with the procedure of Example 3A2 to yield plasmid pL47. A restriction site and function map of plasmid pL47 is presented in FIG. 9 of the accompanying drawings. Plasmid pL47 was transformed into E. coli K12 MO(.lambda..sub.+) in substantial accordance with the procedure of Example 3A3, and the E. coli K12 MO(.lambda..sup.+)/pL47 transformants were identified. Plasmid pL47 DNA was prepared from the transformants in substantial accordance with the procedures of Example 3A1.

DEPR:
Plasmid pPR12 comprises the temperature-sensitive pL repressor gene cI857 and the plasmid pBR322 tetracycline esistance-conferring gene. Plasmid pPR12 is disclosed and claimed in U.S. Pat. No. 4,436,815, issued 13 Mar. 1984. A restriction site and function map of plasmid pPR12 is presented in FIG. 10 of the accompanying drawings.

DEPR:
About 10 .mu.g of plasmid pPR12 were digested with about 50 units of restriction enzyme EcoRI in 200 ml of high-salt buffer at 37.degree. C. for two hours. The EcoRI-digested plasmid pPR12 DNA was precipitated and treated with Klenow in substantial accordance with the procedure of Example 3A5. After the Klenow reaction, the EcoRI-digested, Klenow-treated plasmid pPR12 DNA was recircularized by ligation in substantial accordance with the procedure of Example 3A2. The ligated DNA, which constituted the desired plasmid pPR12.DELTA.R1, was used to transform E. coli K12 RV308 in substantial accordance with the procedure of Example 3A3, except that selection was based on tetracycline (5 ug/ml) resistance, not ampicillin resistance. E. coli K12 RV308 is available from the NRRL under the accession number NRRL B-15624. After the E. coli K12 RV308/pPR12.DELTA.R1 transformants were identified, plasmid pPR12.DELTA.R1 DNA was prepared from the transformants in substantial accordance with the procedure of Example 3A11.

DEPR:
About 10 .mu.g of plasmid pPR12.DELTA.R1 were digested with about 50 units of restriction enzyme AvaI in 200 .mu.l of medium-salt buffer at 37.degree. C. for 2 hours. The AvaI-digested plasmid pPR12.DELTA.R1 DNA was precipitated and treated with Klenow in substantial accordance with the procedure of Example 3A5. After the Klenow reaction, the AvaI-digested, Klenow-treated plasmid pPR12.DELTA.R1 DNA was ligated to EcoR1 linkers (5'-GAGGAATTCCTC-3') in substantial accordance with the procedure of Example 3A2. After the linker ligation, the DNA was precipitated and then resuspended in about 200 .mu.l of high-salt buffer containing about 50 units of restriction enzyme EcoR1. The resulting reaction was incubated at 37.degree. C. for about 2 hours. After the EcoR1 digestion, the reaction mixture was loaded onto an agarose gel, and the .about.5.1 kb EcoR1 restriction fragment was purified in substantial accordance with the procedure of Example 3A6. The

.about.5.1 kb EcoR1 restriction fragment was recircularized by ligation in substantial accordance with the procedure of Example 3A2. The ligated DNA constituted the desired plasmid pPR12AR1. The plasmid pPR12.DELTA.R1 DNA was transformed into E. coli K12 RV308 in substantial accordance with the procedure of Example 3A3, except that selection was based on tetracycline resistance, not ampicillin resistance. After identifying the E. coli K12 RV308/pPR12AR1 transformants, plasmid pPR12AR1 DNA was prepared in substantial accordance with the procedure of Example 3A1. A restriction site and function map of plasmid pPR12AR1 is presented in FIG. 11 of the accompanying drawings.

DEPR:
The .about.2.7 kb PstI-BamHI and .about.1.03 kb EcoRI-BamHI restriction fragments of plasmid pL47 were ligated to the .about.2.9 kb PstI-EcoRI restriction fragment of plasmid pPR12AR1 to construct plasmid pL110, and the ligated DNA was used to transform E. coli K12 RV308 in substantial accordance with the procedure of Examples 3A2 and 3A3, except that tetracycline resistance, not ampicillin resistance, was used as the basis for selecting transformants.

DEPR:
About 1 .mu.g of plasmid pL110 DNA was digested with restriction enzyme NdeI in 20 .mu.l total volume containing 2 .mu.l of lox high-salt buffer (1.0M NaCl; 0.50M Tris-HCl, pH=7.5; 0.10M MgCl.sub.2 ; and 10 mM dithiothreitol) and 3 units of NdeI enzyme for 1 hour at 37.degree. C. The reaction mixture was extracted with phenol/chloroform and the DNA precipitated with ethanol. The NdeI-digested plasmid pL110 DNA was dissolved in 50 .mu.l of 1X Klenow buffer (40 mM KPO.sub.4, pH=7.5; 6.6 mM MgCl.sub.2 ; 1.0 mM 2-mercaptoethanol; 33 .mu.l dATP; 33 .mu.l dCTP; 33 .mu.M dGTP; and 33 .mu.M TTP). Two .mu.l (.about.10 units, New England Biolabs) of the large fragment of E. coli DNA polymerase I, known as Klenow, were added to and mixed with the DNA, and the resulting reaction was incubated at 16.degree. C. for 1 hour. The reaction was terminated by phenol extraction and the DNA conventionally purified. The NdeI-digested, Klenow-treated DNA was then ligated with T4 DNA ligase at 4.degree. C. for 16 hours. The resulting DNA was used to conventionally transform E. coli K12 strain RV308 (NRRL B-15624). Transformants were selected on L-agar plates containing 100 mg/ml ampicillin and plasmids isolated from resistant colonies by the rapid alkaline extraction procedure described by Birnboim and Doly. A plasmid (pL110A in FIG. 13) lacking an NdeI site was selected.

DEPR:
One ml of an overnight culture of E. coli K12 JM109 (E. coli K12 JM101, available from New England Biolabs, can be used instead of E. coli K12 JM109) was used to inoculate 50 ml of L broth, and the resulting culture was incubated at 37.degree. C. with aeration until the O.D..sub.660 was between 0.3 and 0.4. The cells were resuspended in 25 ml of 10 mM NaCl, incubated on ice for 10 minutes, and collected by centrifugation. The cells were resuspended in 1.25 ml of 75 mM CaCl.sub.2 ; a 200 .mu.l aliquot of the cells was removed, added to 10 .mu.l of the ligated DNA prepared above, and incubated on ice for about 40 minutes. The cell-DNA mixture was then incubated at 42.degree. C. for 2 minutes, and varying aliquots (1, 10, and 100 .mu.l) were removed and added to 3 ml of top agar (L broth with 0.5% agar kept molten at 45.degree. C.) that also contained 50 ml of 2% X-Gal, 50 .mu.l of 100 mM IPTG, and 200 .mu.l of E. coli K12 JM109 in logarithmic growth phase. The cell-top agar mixture was then plated on L-agar plates containing 40 mg/ml X-Gal (5-bromo-4chloro-3-indolyl-.beta.-D-thiogalactoside) and 0.1 mM IPTG (isopropyl-.beta.-D-thiogalactoside), and the plates were incubated at 37.degree. C. overnight.

DEPR:
The following morning, several clear, as opposed to blue, plaques were individually used to inoculate 2 ml of L broth, and the resulting cultures were incubated at 37.degree. C. with aeration for 2 hours. The absence of blue color indicates the desired DNA insertion occurred. Then, the cultures were centrifuged, and 200 .mu.l of the resulting supernatant were added to 10 ml cultures (O.D..sub.550 =0.5) of E. coli K12 JM109 growing at 37.degree. C. with aeration. These cultures were incubated for another 30 minutes at 37.degree. C.; then, the cells were pelleted by centrifugation and used to prepare the replicative form of the recombinant phage they contained. Double stranded, replicative form phage DNA was isolated from the cells using a scaled-down version of the procedure described in Example 1. Transformants containing phage

m13Tc3 DNA were identified by restriction enzyme analysis of their phage DNA.

DEPR:
One and one-half ml of an overnight culture of E. coli K12 JM109/m13Tc3 were centrifuged, and 100 .mu.1 of the phage m13Tc3-containing supernatant were used to inoculate a 25 ml culture of E. coli JM109 at an O.D..sub.660 of about 0.4-0.5. The culture was incubated for 6 hours at 37.degree. C. with aeration, at which time the culture was centrifuged and the resulting supernatant, about 20 ml, transferred to a new tube. About 2 ml of a solution containing 20% polyethylene glycol (PEG) 6000 and 14.6% NaCl were added to the supernatant, which was then incubated on ice for 20 minutes.

DEPR:
The DNA pellets were resuspended in 20 .mu.1 Of H.sub.2 O, and 10 .mu.1 of the resulting solution were used to transform E. coli K12 JM109 (E. coli K12 JM101 could also be used) in accordance with the procedure used during the construction of phage m13Tc3, except that no IPTG or X-Gal was added to the plates.

DEPR:
The desired plasmid pL110C was constructed by ligating together 100 nanograms each of the NheI-SalI fragments of pL110A (.about.6.1 kb) and pL110B (.about.422 bp) using conventional procedures. A restriction site and function map of plasmid pL110C is presented in FIG. 13 of the accompanying drawings. The desired plasmid pL110C confers tetracycline resistance to 10 .mu.g/mL tetracycline in E. coli but lacks a BamHI site in the tetracycline resistance-conferring gene.

DEPR:
The DNA was then precipitated and resuspended in 50 .mu.1 of Mung Bean Nuclease Buffer (50 mM Sodium Acetate (pH 5.0), 30 mM NaCl and 1 mM ZnSO.sub.4). One unit of Mung Bean Nuclease (commercially available from New England Biolabs) was added and the reaction was incubated at 30.degree. C. for 30 minutes. The tube was then placed in ice and NaCl was added to 0.2M, then the mixture was phenol/chloroform extracted, ethanol precipitated and resuspended in 10 mM 3.,0 Tris-HCl (pH 8.0). The DNA was then self-ligated and transformed into E. coli cells in substantial accordance with the teaching of Examples 3A3 and 3A4. The resultant plasmid was designated plasmid pCZR111.

DEPR:
Construction of plasmid pCZR126S was accomplished by ligation of the following site components .about.0.28 ug of the 5.8 kb fragment obtained from plasmid pL110 after complete digestion with XbaI and partial digestion with BamHI in a total volume of 2 ul, .about.0.18 ug of the synthetic gene encoding a bovine growth factor derivative which has a 5' termini corresponding to a XbaI site and a 3' termini corresponding to a BamHI site in a total volume of 2.5 ul, 8.75 picomoles of the chemically synthesized XbaI to NdeI linker in 1 ul. The plasmid components were added to 6 ul of 5x ligation buffer: 250 mM Tris-HCl, 50 mM MgCl.sub.2, 5 mM ATP, 5 mM DTT, 25% v/v polyethylene glycol 8,000, pH 7.6, 2 ul of ligase, and 16.5 ul of H.sub.2 O. The ligation mixture was incubated overnight at 16.degree. C. The circularized plasmid pCZR126S was then used to transform E. coli RV308 cells in substantial accord with the method of Example 3A3. A restriction site and function map of plasmid pCZR126S is presented in FIG. 14 of the accompanying drawings.

DEPR:
About 2.5 .mu.1 of the vector DNA was mixed with 12 .mu.1 of the purified ACB-proinsulin gene fragment from above, 4 .mu.1 of 10 mN ATP, 0.5 .mu.1 of 1M dithiothreitol, 5 .mu.1 of 10X ligase buffer (500 mM tris-HCl, pH=7.6, 100 mM MgCl.sub.2), 26 .mu.1 of water and 0.5 .mu.1 of T4 DNA ligase (Pharmacia, Inc., 800 Centennial Avenue, Piscataway, N.J. 08854, 3.5 units). The reaction was incubated at 4.degree. C. for 16 hours. The ligated mixture was diluted with 50 mL of 10 mM tris-HCl (pH=7.6) and 3 .mu.1 of 1M CaCl.sub.2 and then subsequently transformed into E. coli K12 RV308 in accordance with the teaching of Example 3A3 above. The cells were plated on T4 agar plates supplemented with 5 .mu.g/ml tetracycline and incubated overnight at 32.degree. C.

DEPR:
Scale-up production of cells for extraction and purification of recombinant ACB-proinsulin was accomplished using a BioFlo benchtop fermenter (commercially available from New Brunswick Scientific Co., Inc., P.O. Box 986, 44 Talmadge

Identification of the products of bacteriophage P22 genes, including a new late gene

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We report the identification of the products of several bacteriophage P22 genes by SDS-polyacrylamide gel electrophoresis. The products of P22 early genes int, erf, 17, 18, 12, and 23 are identified by their presence in radioactively labeled lysates of cells infected with P22 wild-type phage and absence in lysates of cells infected with phages carrying amber mutations in these genes. The products of the two P22 late genes essential for host cell **lysis** , **genes 13** and **19** , are also identified using amber mutants. We also report the isolation and characterization of amber mutants defining a previously unknown P22 late gene, gene 14, and identify the protein product of this new gene. P22 gene 14 amber mutants, like coliphage T4 gene 40 nonsense mutants, have the unusual property that they display a conditional-lethal phenotype only at high temperature. The apparent molecular weights of these and previously identified P22 gene products have been determined. The products of all known essential P22 genes, with the exception of gene 24, have now been identified by SDS-polyacrylamide gel electrophoresis.

Genetic and DNA mapping of the late regulation and lysis genes of Salmonella bacteriophage P22 and coliphage lambda.

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Genetic and DNA heteroduplex analyses of lambda imm22 hybrid phages were used to compare the Salmonella bacteriophage P22 and coliphage lambda genes which control late gene regulation and lysis. Homologous DNA sequences were correlated with P22 gene 23 and lambda gene Q (late gene regulation) and with P22 gene **13** and lambda **gene** S (**lysis** control). Nonhomologous DNA sequences were correlated with P22 gene 19 and lambda gene R (lysozyme and endolysin) and with the region encoding the P22 alpha and lambda 6S transcripts.

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.
Descriptors: *Bacteriophage lambda--Genetics--GE; *Genes, Viral; *Salmonella Phages--Genetics--GE; Chromosome Deletion; Chromosome Mapping; DNA, Viral--Genetics--GE; Endopeptidases--Genetics--GE; Gene Expression Regulation; Muramidase--Genetics--GE; Recombination, Genetic; Sequence Homology, Nucleic Acid; Virus Replication
CAS Registry No.: 0 (DNA, Viral)
Enzyme No.: EC 3.2.1.17 (Muramidase); EC 3.4.- (Endopeptidases); EC 3.4.99.- (endolysin)

Posttranscriptional modulation of bacteriophage P22 scaffolding protein gene expression.

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The bacteriophage P22 late operon contains 2 genes whose products are required for cell **lysis** and **13 genes** whose products are involved in the morphogenesis of the phage particle. This operon is under the positive control of the phage gene 23 product and is thought to have a single promoter. The expression of one of these late genes, the scaffolding protein gene, is autogenously modulated independently from the remainder of the late genes. When unassembled, scaffolding protein turns down the rate of synthesis of additional scaffolding protein, and when it is assembled into phage precursor structures, it does not. Experiments presented here show (i) that the mRNA from the scaffolding protein gene is functionally threefold more stable when most of the scaffolding protein is assembled than when it is unassembled and (ii) that no new promoter near the scaffolding protein gene is activated at the high level of synthesis. These data support the model that this autogenous modulation occurs at a posttranscriptional level. We also observed that another message, that of coat protein, appears to become increasingly stable with time after phage infection.

Global suppression of protein folding defects and inclusion body formation.

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THE FOLDING OF POLYPEPTIDE CHAINS INTO THEIR NATIVE states requires the selection of one conformation out of an enourmous ensemble of sterically available but incorrect conformations [1]. During the in vitro refolding of polypeptide chains formation of incorrect aggregated states frequently competes with folding into the native conformation [2-4]. Polypeptide chains synthesized within both prokaryotic and eukaryotic cells are also lost to aggregation, particularly at higher temperatures [5-7]. One important function of the heat shock proteins is probably interaction with folding intermediates to chaperone them through the productive folding pathway by preventing aggregation [8-10]. The expression of the protein product of cloned genes in foreign hosts often results in accumulation of the newly synthesized polypeptide chains in an aggregated nonnative state or inclusion body [11, 12]. The few systematic studies on this subject suggests that aggregates are off-pathway polymeric structures derived, both in vitro and in vivo, from folding intermediates in the productive pathway [13, 14]. An unanswered question is to what extent these aggregation processes reflect the folding instructions encoded in the amino acid sequence.

Mutational analysis of intracellular chain folding. Mutational and comparative sequence studies suggest that the residues determining chain conformation are dispersed throughout the sequence [15-17]. At least two kinds of information are contained in these critical sequences. One kind includes residues stabilizing the native state [18-21]. A second kind of sequence information probably ensures that the newly synthesized polypeptide chain forms and passes through the correct intermediate conformations [22-25]. This includes both auxiliary sequences--such as propeptides, registration peptides, and signal sequences [26]--and residues within the mature chain [27-29].

We have been engaged in efforts to identify residues and sequences controlling the conformation of intermediates and perhaps also off-pathway steps, initially through the isolation and characterization of temperature-sensitive folding (tsf) mutations in the P22 tailspike protein [6, 17, 27, 30]. The tailspike protein of Salmonella phage P22 is a homotrimer of three 666 amino acid chains [31, 32] whose secondary structure is dominated by [beta] sheet, as revealed by Raman spectroscopy [33, 34]. Although normally bound to the mature virion, native tailspikes accumulate as soluble structural proteins when capsid assembly is blocked by mutation.

As a result of a number of properties of the thermostable tailspike and of phage-infected cells, the cytoplasmic folding and aggregation pathway of tailspike polypeptide chains can be studied in vivo [23, 27]. The pathway invovles only conformational transitions with no known covalent modifications. Single chain and triple chain partially folded intermediates have been characterized in lysates of infected cells. Although the native tailspike is thermostable, the single chain intermediate is thermostable and partitions between the productive pathway and nonnative, aggregated forms of a elevated temperatures [6, 27, 35].

Chains synthesized at high temperatures can reenter the productive pathway if shifted to permissive temperature early enough [6, 27, 30]. However, the aggregated chains are kinetically trapped and are not recoverable.

After denaturation of purified tailspikes with acid urea, the polypeptide chains can be refolded in vitro by dilution into physiological buffer [36]. The in vitro refolding reaction is also sensitive to temperature with off-pathway aggregation dominating above room temperature.

Temperature-sensitive folding mutations prevent the newly synthesized chain within infected cells from reaching the native state at high

temperatures [30]. They interfere with the folding of the single chain intermediate, preventing it from attaining the confrontation necessary for association into the protrimer intermediate (Fig. 1) [27]. The mutant chains are not degraded, but aggregate into inclusions bodies [6].

The failure of the tsf mutations to reach their native state at high temperatures is not due to lowered stability or activity of the native state. Once the native state is attained at permissive temperature, the mutant proteins are as stable as the wild type when the temperatures are raised, with melting temperatures near 88[degrees]C [34, 35, 37]. The physiological functions of the native forms of the mutant proteins, such as binding to phage heads, and adsorption to bacterial cells are not distinguishable from wild type [35].

Characterization of the native forms of the mutant proteins suggested that most tsf mutations are in sequences resembling those reported for turns and located at the protein surface in the native conformation [17, 38]. These mutations may prevent correct [beta]-turn and [beta]-sheet information in the critical folding intermediates at restrictive temperature [38, 39]. Thus the tsf sites identify positions in the chain important for the folding pathway, but making only minor contributions to the stability of the native state.

Mutants defective in tailspike stability and head binding to the phage head have also been described by Berget and co-workers [40]. They isolated a second site conformational suppressor that corrects such a defect in stability and head binding. This suppressor appears to alleviate the loss of a critical salt bridge in the [NH.sub.2]-terminal region of the native protein.

Fane and King [41] isolated second-site suppressors which alleviated the folding defects conferred by missence and tsf substitutions. Fane et al. [42] found that two second-site mutations were repeatedly isolated; they are V331[right arrow]A and A334[right arrow]V [43]. These two mutations suppressed absolute folding defects and temperature-sensitive folding defects at a number of sites in the chain, indicating that the suppressors operate through some global or general mechanism [42].

A well-characterized tailspike tsf mutant suppressed by the amino acid substitutions V331[right arrow]A and A334[right arrow]V in the tailspike protein is Tsf G244[right arrow]R (formerly tsfH304; [30, 44]). Synthetic peptides with the wild-type form [beta] turns in solution, while the mutant substitution decreases this tendency fourfold [39]. To investigate the mechanism of the suppression we examined the intracellular maturation and the properties of the native state for Su A334[right arrow]V:Tsf G244[right arrow]R, Tsf G244[right arrow]R, and Su A334[right arrow]V chains.

Stability and activity of mature protein, carrying global suppressor substitutions. The growth curves of phages carrying suppressor mutations in their genomes were not distinguishable from growth curves of wild type, an indication that the amino acid substitutions did not interfere with the various functions of the native state of the tailspike. To examine these mutant proteins more carefully, we purified the native forms of the Tsf G244[right arrow]R, Su A334[right arrow]V: Tsf G244[right arrow]R, Su V331[right arrow]A and Su A334[right arrow]V proteins [38]. A native gel separation of the purified mature forms of the mutant proteins is shown in Fig. 2. Since the proteins carrying the

[TABLE DATA OMITTED]

Tsf G244[right arrow]R substitution have an extra positive charge, they migrate more slowly through the gel [38]. The presence of the suppressor mutation had no effect on the mobility of the native forms of the tailspike.

The melting temperatures ([T.sub.m]) were determined by scanning calorimetry as described earlier for the native trimeric forms of the wild-type and tsf mutant proteins [37]; the tsf mutations by themselves had little effect on the [T.sub.m]. The suppressor mutations have very little effect on the melting temperatures of the proteins, alone or in combination with Tsf G244[right arrow]R (Table 1). These mutations alter neither the ability of the proteins to bind to phage heads nor their infectivity properties. Thus, the suppressor mutations do not have detrimental effects on the function and stability of the native product.

Effect of suppressor substitutions on polypeptide chain folding and

aggregation. To determine if the suppression was occurring during polypeptide chain folding and association, we examined the intracellular synthesis, folding, and assembly of tailspike chains into the native SDS-resistant tailspike. For these experiments we constructed phage strains that carried amber mutations in gene 5 encoding the major coat protein and in **gene 13** controlling **lysis** . In the restrictive Salmonella host cells infected with these strains the tailspike is one of the major polypeptide chains synthesized [30, 45].

We exposed infected cells to a 90-second pulse of [sup.14.C]-labeled amino acids in order to label newly synthesized polypeptide chains. The folding and assembly of the chains were stopped by rapid chilling, and the concentrated cells were frozen and thawed at 4[degrees]C to promote lysis. The resulting lysates were fractionated by centrifugation. The sediment and supernatant fractions were assayed for tailspike chains by SDS-gel electrophoresis without prior heating of the samples. Under these conditions, the mature trimeric tailspike remained native in SDS gels, whereas the ensemble of folding and assembly intermediates dissociated into complexes of SDS and polypeptide chains [31]. The tailspike chains that sedimented at low speed and were dissociable in SDS without heating are defined as the aggregated inclusion body state [6]. These procedures distinguished three classes of newly synthesized tailspike species, namely, soluble partially folded intermediates, aggregated inclusion body forms, and SDS resistant native trimers.

The rates of synthesis of mutant and wild-type chains were similar in the lysates. The wild-type chains (Fig. 3, lower right) formed soluble partially folded intermediates, which partitioned between native tailspike and aggregated chains. At 39[degrees]C, the temperature of this experiment, about 35 percent of the chains reached the native state, while the remaining 65 percent ended up in the aggregated inclusion body state.

In the infected cells with the tsf mutant (Fig. 3, upper left) at restrictive temperature, a soluble partially folded species formed, but this species failed to mature to tailspike trimers. All of the chains aggregated into the inclusion body state [6].

The presence of the Su A334[right arrow]V substitution altered the behavior of the Tsf chains. Thirty five percent of the double mutant chains matured to the native tailspike, approaching what was found with the wild type. The aggregated fraction was correspondingly less (Fig. 3, upper right).

Chains carrying the suppressor alone (Su A334[right arrow]V) matured more efficiently than wild type, yielding 60 percent of the chains in the native conformation compared with 35 percent for wild-type chains. Again, this increased efficiency appears to result from the decreased fraction of aggregated chains (Fig. 3, lower left). The kinetics of the disappearance of the soluble intermediates was similar for the wild type, suppressor, tsf, and suppressor:tsf infected cells. The suppressor mutations seem to act by altering the partition of the soluble intermediate between the aggregated state and the productive pathway. At the permissive 30[degrees]C temperature, 60 percent of the Tsf chains matured to native tailspikes, while more than 90 percent of the wild-type, suppressor, and suppressor:Tsf chains matured to the native state. Qualitatively similar results were found with the Su V331[right arrow]A suppressor and Tsf G244[right arrow]R, and with both suppressors combined with a different well-characterized tsf mutation, Tsf E309[right arrow]V [38].

The tsf mutant chains do not reach the protrimer species at restrictive temperature and thus appear to be blocked before chain-chain association [27]. We examined the ability of chains carrying only the suppressor substitution to rescue other chains carrying the Tsf substitution. Cells were coinfected with phage strains carrying separately the Tsf G244[right arrow]R and Su A334[right arrow]V substitutions. The altered electrophoretic mobility of Tsf G244[right arrow]R and the wild-type mobility of Su A334[right arrow]V made it possible to distinguish the formation of mutant, suppressor, and hybrid trimers in a mixed infection experiment [31, 38]. At permissive temperature, both strains formed native homotrimers, as well as hybrid trimers (Fig. 4).

At restrictive temperature only suppressor trimer formation was observed, indicating that chains carrying suppressors could not rescue other chains carrying Tsf substitutions. The simplest explanation for this

is that suppression occurred through the monomeric folding intermediate, prior to chain-chain association.

Mechanisms of inclusion body formation. Although aggregation has often been considered a nonspecific process, the experimental evidence from both in vivo and in vitro studies indicate otherwise; aggregates are formed from specific partially folded intermediates [2, 3, 13, 14, 46]. Thus, the interactions in the aggregate are likely to resemble intrachain interactions between units of secondary structure found in the folded proteins. For example, in gelatin the bonds holding the chains together are locally triple helical as in native collagen, but the chains are out of register [47]. Our data establish that a single, relatively subtle amino acid substitution can inhibit the intracellular aggregation of the tailspike folding intermediates. These data confirm that the steps leading to inclusion body formation involve specific regions of the amino acid sequence.

Aggregation of the tailspike chains may well involve interactions between specific sites on the folding intermediates. In that case the suppressor substitution may be disrupting these interactions directly, thus identifying the aggregation site on the folding intermediate. Alternatively, the substitutions may be influencing the aggregation process through less localized effects on the conformation of the folding intermediates. Lee, Koh, and Yu [48] have substituted different amino acids at the 331 and 334 sites and found that the ability to suppress tsf mutants is found only with a limited set of substitutions.

The local sequence of the suppressors

Ser - Tyr - Gly - Ser - Val [up arrow] (suAla) - Ser - Ser - Ala [up arrow] (suVal) - Gln - Phe - Leu - Arg

reveals no singular features. High serine content is associated with [beta] strands and with flexible regions. The location of the tsf mutation, S333[right arrow]N, suggests that the local conformation in the native state might be a surface [beta] turn [17].

If tsf mutations decrease the thermal stability of a monomeric folding intermediate, the suppressors may confer increased stability to this critical intermediate species. Alternatively, they could kinetically influence the partition of the critical intermediates between intramolecular and intermolecular fates.

The purified Tsf, suppressor, and suppressor:Tsf proteins have also been refolded from the fully denatured state according to the procedures described by Seckler et al. [36]. At 36[degrees]C the presence of the 334 substitution significantly increased the recovery of the native trimeric tailspikes, compared to the very low yield of the Tsf substitution alone [49]; although it is not known if the efficacy of the suppressor in vitro corresponds to the in vitro efficiency, the result does show that the in vitro maturation of tailspikes is directly affected by the amino acid substitution, in the absence of any cellular factors.

The in vitro results do not exclude the possibility that the in vivo aggregation of the wild-type chains at high temperature could be due to failure of, or poor interaction of, folding intermediates with a molecular chaperone. This would explain the thermolability of the single chain folding intermediate in the wild-type pathway. The suppressor mutation would create, improve, or restore a site of chaperonin binding or recognition. Van Dyk, Gatenby, and La Rossa [50] have reported evidence that some tailspike tsf mutants can be rescued by overproduction of GroE chaperonin. The role of host cell chaperonins is under investigation [51].

Observations from Raman spectroscopy [33], x-ray diffraction, and electron microscopy indicate that the chains are in a cross-[beta] conformation, with short lengths of [beta] strand running orthogonal to the long axis of the tailspike. If the correct intrachain interactions are disturbed past a turn site, the intermediate may transiently accumulate as partially formed [beta]-strand structures. Aggregation of those intermediate structures would subsequently occur through "out-of register" interchain strand interactions.

Whatever the mechanism, these single amino acid substitutions direct the polypeptide chain away from aggregation traps, without altering the activity and stability of the mature protein. This suggests that it is possible to optimize folding pathways without affecting the desired properties of the final native state. The isolation of such mutations in

other proteins may permit the engineering of scientifically or industrially important proteins to improve their recovery from heterologous hosts or in downstream processing [52].

Implications for the protein folding grammar. As a result of the flexibility of polypeptide chains, a large ensemble of conformations is sterically available during the folding process [1]. Successful negotiating of the folding pathway to reach the native conformation requires avoiding the many conformations that would lead to incorrect chain interactions. Many of the aggregated states are liable to be kinetic traps corresponding to local energetic minima.

We presume that the sites or sequences generated by these suppressor mutations identify a class that is well represented in the wild-type sequence of the tailspike and in other polypeptide chains. Mutations isolated by Wetzel et al. that altered inclusion body formation of human interferon may be of this character [52]. Such sequences may have only a limited role in stabilizing the native state. Indeed, their main role could be kinetically blocking off-pathway interactions or destabilizing incorrect conformations. Such residues represent a third kind of sequence information, in addition to those described above.

In comparing globin amino acid sequences from more than 260 species, Bashford, Chothia, and Lesk [16] identified more than 30 solvent-exposed positions associated with the protein surface at which hydrophilic residues were conserved. Some of these sites may represent the kinds of residues reported here, whose role is not stabilization of the native state, but destabilization of incorrect states--for example, helices packed through the wrong faces. The concept of local sequences that destabilize incorrect structures has also emerged out of efforts to design a chain that would fold into a specific conformation where residues were incorporated to block incorrect interactions [53]

The existence of sequences whose function is to block incorrect structures makes it easier to understand why it may not be possible to deduce the folding grammar simply by the comparison of the native state and the primary sequence.

Note added in proof: While this paper was in press Tsai et al. [54] reported the isolation of temperature-sensitive mutations of the human receptor-like protein tyrosine phosphate, as well as isolation of suppressors of the temperature-sensitive mutations. The suppressor increased the yield of correctly foled protein in Escherichia coli and may be functioning like the suppressors described here.

REFERENCES AND NOTES

[1] G. N. Ramachandran and V. Sasisekharan, Adv. Prot. Chem. 23, 283 (1968).

[2] J. London, C. Skrzynia, M. Goldberg, Eur. J. Biochem. 47, 409 (1974).

[3] G. Zettlmeissl, R. Rudolph, R. Jaenicke, Biochemistry 18, 5567 (1979).

[4] A. Mitraki, J.-M. Betton, M. Desmadril, J. Yon, Eur. J. Biochem. 163, 29 (1987).

[5] M.-J. Gething, K. Mc Cammon, J. Sambrook, Cell 46, 939 (1986).

[6] C. Haase-Pettingell and J. King, J. Biol. Chem. 263, 4977 (1988).

[7] S. M. Hurtley and A. Helenius, Annu. Rev. Cell. Biol. 5, 277 (1989).

[8] S. M. Hemmingsen et al., Nature 333, 330 (1988).

[9] J. E. Rothman, Cell 59, 591 (1989).

[10] B. Goloubinoff, A. A. Gatenby, G. Lorimer, Nature 337, 44 (1989).

[11] F. A. O. Marston, Biochem. J. 240, 1 (1986); G. Georgiou; J. N. Telford, M. Schuler, D. B. Wilson, Appl. Environ. Microbiol. 52, 1152 (1986).

[12] R. Wetzel, L. J. Perry, M. G. Mulkerrin, L. M. Randall, in Protein Design and the Development of New Therapeutics and Vaccines, J. Hooke and G. Poste, Eds. (Plenum, New York, 1990), pp. 79-115.

[13] A. Mitraki and J. King, Bio Technology 7, 690 (1989).

[14] J. L. Cleland and D. I. C. Wang, Biochemistry 29, 11072 (1990).

[15] J. King, Biotechnology 4, 297 (1986).

[16] D. Bashford, C. Chothia, A. M. Lesk, J. Mol. Biol. 196, 199 (1987).

[17] R. Villafane and J. King, J. Mol. Biol. 204, 607 (1988).

[18] D. P. Goldenberg, Annu. Rev. Biophys. Biophys. Chem. 17, 481 (1988).

[19] D. Shortle and B. Lin, Genetics 110, 539 (1985). D. Shortle, J. Biol. Chem. 264, 5315 (1989).

[20] T. Alber, Annu. Rev. Biochem. 58, 765 (1989).

[21] J. U. Bowie, J. Reidhaar-Olson, W. A. Lim, R. T. Sauer, Science 247, 1306 (1990).

[22] T. E. Creighton, Prog. Biophys. Mol. Biol. 33, 231 (1978).

[23] D. Goldenberg and J. King, Proc. Natl. Acad. Sci. U.S.A. 79, 3403 (1982).

[24] J. King et al., in Protein Folding, Deciphering the Second Half of the Genetic Code; L. M. Gierasch and J. King, Eds. (AAAS, Washington, DC, 1990), pp. 225-239.

[25] P. S. Kim and R. L. Baldwin, Annu. Rev. Biochem. 59, 631-660 (1990).

[26] P. Walter, R. Gilmore, G. Blobel, Cell 38, 5 (1984).

[27] D. P. Goldenberg, D. H. Smith, J. King, Proc. Natl. Acad. Sci. U.S.A. 80, 7060 (1983).

[28] M.-H. Yu and J. King, ibid. 81, 6584 (1984).

[29] D.P. Goldenberg, R. W. Frieden, J. A. Haack, T. B. Morrison, Nature 338, 127 (1988).

[30] D. H. Smith and J. King, J. Mol. Biol. 145, 653 (1981).

[31] D. P. Goldenberg, P. B. Berget, J. King, J. Biol. Chem. 257, 7864 (1982).

[32] R. T. Sauer, W. Krovatin, A. R. Poteete, P. B. Berget, Biochemistry 21, 5811 (1982).

[33] D. Sargent, J. M. Benevides, M.-H. Yu, J. King, G. J. Thomas, Jr., J. Mol. Biol. 199, 491 (1988).

[34] G. J. Thomas, Jr., R. Becka, D. Sargent, M.-H. Yu, J. King, Biochemistry 29, 4181 (1990).

[35] D. Goldenberg and J. King, J. Mol. Biol. 145, 633 (1981).

[36] R. Seckler, A. Fuchs, J. King, R. Jaenicke, J. Biol. Chem. 264, 11750 (1989); A. Fuchs, C. Sciderer, R. Seckler, Biochemistry, in press.

[37] J. Sturtevant, M.-H. Yu, C. Haase-Pettingell, J. King, J. Biol. Chem. 264, 10693 (1989).

[38] M.-H. Yu and J. King, ibid. 263, 1424 (1988).

[39] A. N. Stroup and L. M. Gierasch, Biochemistry 29, 9765 (1990).

[40] P. A. Maurides, J. J. Schwarz, P. B. Berget, Genetics 125, 673 (1990).

[41] B. Fane and J. King, Genetics 127, 263 (1991).

[42] B. Fane, R. Villafane, A. Mitraki, J. King, J. Biol. Chem., in press.

[43] Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

[44] D. H. Smith, P. B. Berget, J. King, Genetics 96, 331 (1980).

[45] P. B. Berget and A. P. Poteete, J. Virol. 34, 234 (1980).

[46] D. N. Brems, S. M. Plaisted, E. W. Kauffman, H. A. Havel, Biochemistry 25, 6539 (1986); G. A. Bowden and G. Georgiou, J. Biol. Chem. 265, 16760 (1990).

[47] W. F. Harrington and N. V. Rao, Biochemistry 9, 3714 (1970).

[48] S. C. Lee, H. Koh, M.-H. Yu, personal communication.

[49] A. Mitraki and R. Seckler, unpublished results.

[50] T. K. Van Dyk, A. A. Gatenby, T. A. LaRossa, Nature 342, 451 (1989).

[51] S. Sather and J. King, unpublished results.

[52] R. Wetzel, L. J. Perry, C. Veilleux, Biotechnology, in press.

[53] J. Richardson and D. Richardson, Science 240, 4859 (1988); M. H. Hecht, D. C. Richardson, J. S. Richardson, ibid. 249, 884 (1990).

[54] A. Y. M. Tsai, M. Itoh, M. Streuli, T. Thai, H. Saito, J. Biol. Chem. 266, 10534 (1990).

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CAPTIONS: Thermostability of purified native forms of proteins. (table);

Pathway of intracellular folding. (chart); Folding and intracellular aggregation. (graph)

SPECIAL FEATURES: illustration; table; chart; photograph; graph
DESCRIPTORS: Mutation (Biology)--Research; Protein folding--Research; Protein engineering--Research
FILE SEGMENT: MI File 47

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DIALOG(R)File 399:CA SEARCH(R)

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Conservation of a dual-start motif in P22 lysis gene regulation
AUTHOR(S): Nam, Kiebang; Blasi, Udo; Zagotta, Michelle T.; Young, Ry
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SECTION:
CA203002 Biochemical Genetics
IDENTIFIERS: bacteriophage P22 lysis gene 13 protein, phage lambda lysis gene S product
DESCRIPTORS:
Gene and Genetic element,microbial, S...
for cell lysis, of bacteriophage .lambda., dual start motif in regulation of
Gene and Genetic element,microbial, 13...
for cell lysis, of bacteriophage P22, dual-start motif in regulation of
Virus,bacterial, lambda...
lysis gene S of, regulation of, dual-start motif in
Virus,bacterial, P22...
lysis gene 13 of, regulation of, dual-start motif in
Proteins,specific or class, gene S...
of bacteriophage .lambda., regulation of, cell lysis in relation to
Proteins,specific or class, gene 13...
of bacteriophage P22, regulation of, cell lysis in relation to
Deoxyribonucleic acid sequences...
of gene S, of bacteriophage .lambda.
Deoxyribonucleic acid sequences...
of lysis gene 13, of bacteriophage P22

Road, Edison, N.J. 08817). Five liters of 2X TY broth containing 5ug/ml tetracycline (obtained from Sigma Chemical Co.) plus 1.0 ml of antifoam SAG 5693 (commercially available from Union Carbide, Specialty Chemical Division, Danbury, Conn. 06817-0001) was inoculated with 100 ml of bacterial culture of E. coli K12 RV 308 cells containing the pRB182 plasmid were grown overnight at 30.degree. C. Cells were grown at 32.degree. C. until the end of the exponential growth phase. Next, glucose and case-amino acids were added to concentrations of 0.2% and 0.1% respectively and the temperature shifted to 42.degree. C. to induce protein synthesis. The cells were harvested from the growth medium two hours post-induction by centrifugation at 500 g for 10 minutes at 4.degree. C. The supernatant was discarded and the pellet was washed once with ice cold TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

DEPR:
43.5 g of E. coli cells (wet weight) were suspended in 400 ml of 20 mM Tris-HCl pH=7.6 containing 10 mM EDTA, 1 mM PMSF, 10% sucrose and 100 ug/ml lysozyme. The mixture was stirred vigorously for 1.5 hours at room temperature (approx. 25.degree. C.) , chilled on ice for 30 minutes, and the cells disrupted by sonication. The granules were collected by centrifugation at 2200g at 4.degree. C. for one hour. The granules were then washed with 20 mM Tris-HCl, 1M NaCl, pH=7.6. The granules were dissolved with stirring in 200 ml of 20 mM Tris-HCl, 8M guanidine-HCl, pH=8.8. Next, 7g of Na.sub.2 SO.sub.3 and 5g of Na.sub.2 S.sub.4 O.sub.6 were added and the solution was stirred for three hours at room temperature. Following centrifugation, the supernatant was dialyzed using a 1000 MWCO dialysis bag (commercially available from Spectrum medical Industries, Inc., Los Angeles, Calif. 90060) against three changes of 2 liters of 10 mM ammonium acetate, pH=7.4. A precipitate developed which was collected by centrifugation at 2200g, 40.degree. C., 1 hour. The supernatant was acidified to pH=3.6 with 6N HCl and the resulting precipitate collected and added to the precipitate from the dialysate.

DEPC:
Construction of E. coli K12 MO (.lambda..sup..+-.)/pKC283PX

DEPC:
Construction of E. coli K12 MO(.lambda..sup..+-.)/pKC283-L

DEPC:
Construction of E. coli K12 MO(.lambda..sup.+)/pKC283-LB

DEPC:
Construction of E. coli K12 MO(.lambda..sup.+)/pL32

DEPC:
Construction of E. coli K12 MO(.lambda..sup..+-.)/pL47

DEPC:
Construction of E. coli K12 RV308/pPR12AR1

DEPC:
Construction of E. coli K12 RV308/pL110

DEPC:
Construction of E. coli K12 RV308/pL110C

DEPC:
Example 3.A.10.a Construction of E. coli K12 RV308/pL110A

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 5185431 A

L12: Entry 4 of 4 File: USPT Feb. 9, 1993

DOCUMENT-IDENTIFIER: US 5185431 A
TITLE: Recombinant natural killer cell activator

BSPR:
Furthermore, a host transformed with said expression plasmid also constitute the present invention like the abovementioned cDNA and expression plasmid As the host, Escherichia coli, yeasts and animal cells such as BHK cells and CHO cells may be employed.

BSPR:
An example of an E. coli strain carrying the cDNA is one discriminated as XL1-Blue/pNK 8308 B which will be described in Example 1. The strain has been deposited with Fermentation Research Institute as FERM P-10161, now transferred to the international deposit, FERM BP-2468.

DEPR:
pBRD5001 is obtained by replacing the vector portion of the lymphotoxin expression plasmid pTT5001 (Japanese Patent Application 272034/1987 by pBR322 d-rop (Japanese Patent Application 272034/1987 and is expected to stably retain a large copy number of the plasmid. This expression vector has a trc promoter and rrnB terminator derived from pKK233-2 (mfd. by Pharmacia Fine Chemicals Co.).

DEPR:
pPL9-5001 was constructed from pBRD5001, by replacing the promoter with the PL promoter of bacteriopharge .lambda.. pPL- (mfd. by Pharmacia Fine Chemical Co.) was cleaved with EcoRI and HpaI to isolate fragment of approximately 470 bp containing PL promoter. This fragment was cleaved with Hae III to obtain an approximately 265 bp fragment and this fragment was ligated along with the DNA fragment of the following synthetic SD sequence; ##STR2## to EcoRI-BglII cleaved pUG131 plasmid the plasmid obtained by replacing the polylinker of pUC13 (mfd. by Pharmacia Fine Chemicals Co.) for the polylinker of M13 tg131 (mfd. by Amercham Co.) (Japanese Patent Application 272034/1987)] to generate pPL9. pBRD5001 was cleaved with EcoRI and Bgl II to remove an approximately 300 bp fragment containing trc promoter and the fragment (PL promoter+SD) of about 280 bp produced by cleaving pPL9 with EcoRI was inserted to create pPL9-5001.

DEPR:
This variant NKAF cDNA was excised with Afl II to create the NKAF fragment without signal sequence. A synthetic DNA linker having a Bgl II-cohesive end and an initiation codon followed by a sequence coding for Leu His was inserted upstream of the NKAF cDNA fragment and ligated to the vector fragment (Bgl II - SalI) of pEH7084 (Japanese Patent Application 253302/1988)), creating pENK702 (FIG. 13). In this plasmid, the cDNA coding for the mature NKAF is connected just downstream of the trc promoter of pENK702 via the Bgl II site. A Bgl II - Pvul vector fragment of pBRD5001 and a Bgl II - Pvul NKAF cDNA fragment of pENK702 was then ligated thereto to create pBRD702 (FIG. 14).

DEPR:
An E. coli strain, N4840 CI857, has a temperature sensitive mutation repressor and induction of the PL promoter is achieved by a high-temperature shift.

DEPC:
Example Added to NKAF Patent Application (NKAF Expression in Escherichia coli)

DETL:
TABLE 13 ______________________________________ NKAF expression in E. coli N 4840 Time after temperature shift Plasmid 0 1.5 2 5 7.5 h ______________________________________ pNK8001 3 5 26 33 19 ng/ml pPL9-5001 2 -- -- -- 3 ______________________________________

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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USPT	4436815.pn.	1	L13
USPT	l11 and l9	4	L12
USPT	l8 and (ptrc or trc)	4	L11
USPT	l8 same (ptrc or trc)	0	L10
USPT	l8 and (coli or salmonella)	84	L9
USPT	l7 same l6	84	L8
USPT	cI857	407	L7
USPT	pr or pl or pola	46487	L6
USPT	l4 and pola	2	L5
USPT	dap-less	15	L4
USPT	l2 and asd	1	L3
USPT	l1 and pola	1	L2
USPT	4968619.pn.	1	L1

West